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The satisfactory killing, staining and fixing of protozoa on glass slides is somewhat difficult and requires considerable patience and experience to obtain good results. Distortion of the body of the organisms is almost unavoidable. Most bacteriological stains used in connection with the dry or flame methods of fixing are not satisfactory except in the case of the minute flagellates. The killing agent must act rapidly or distortion will result. Osmic vapor or 2 per cent solution of the acid, potassium iodide, bichloride of mercury (saturated solution) to which has been added 1 per cent acetic acid, and picrosulfuric acid were the best killing reagents used. Intra-vitam staining with dilute solutions of methyl green, methylene blue or Congo red gave very good results. The Romanowsky stain as modified by Morgan (24) was tried with good results but consumed too much time for routine work. Iron haemotoxin gave good results as a nuclear stain, while for staining cilia, tannic acid was good. In the case of very active organisms it was found that observation could be much simplified by placing a few strands of cotton on the slide to restrict motility. Dilute agar solution is also useful for this purpose. For further information on the subject of staining and fixing protozoa the reader is referred to a short, concise article by Hargitt (11).

DIRECT EXAMINATION OF SOIL FOR PROTOZOA

All samples inoculated into media for study were previously studied microscopically to detect trophic protozoa. For this examination the soil sample was dampened with water, placed on a slide and examined for about 5 minutes. If no living cells were detected in that time, the sample was called negative. In but two soils collected were living protozoa found and then only in very small numbers. Samples of soil taken near ditches or ponds practically always showed living specimens, as did samples collected in low places where there was standing water after heavy rains. These latter samples are not included in the number of soils classed as normal and on which our work was based.

PRESENT STATUS OF OUR KNOWLEDGE CONCERNING THE CHARACTER OF THE SOIL FAUNA

It is interesting to speculate for a moment upon the manner in which protozoa are spread in soil. Leeuwenhoek (19) as early as 1677 found and described several species of protozoa from rain-water. The rain is therefore one of the means by which cysts were originally distributed in soils. Ehrenberg (8, 9) in 1837 mentions that he found large numbers of protozoa in *rich* soils. Since his time, Rosenburg-Lipinsky (28), Wolff (38), Cunningham and Lohnis (5), Martin (21), Waksman (34) and others mention the existence of a considerable soil fauna. No specific reference was found suggesting a plausible explanation for the presence in soil of such large numbers of species and protozoan cells. Miquel (23) showed that protozoa and algal spores or cysts were common in air and rain-water even on the tops of the highest mountains of France. It is suggested that the air, rain, snow, winds, etc., disseminate these or ganisms over all the land. Up to the present day the validity of the old "egg and germ" theory of distribution of species by the elements mainly, has not been seriously questioned. All biologists know that protozoa are abundant on grass-stems, bark of trees, among mosses and lichens and even other places where their presence would not be expected because of the lack of moisture. Leidy (20) in his excellent treatise on rhizopods states, "Although essentially aquatic, they (rhizopods) occur wherever there is moisture, including damp rocks and soils, among vegetation, on the barks of trees, and even in the crevices of walls and pavements in cities." Pleas (25) found many living species of algae, protozoa and rotifers among lichens collected in mid-winter from dry rocks and fences. He found organisms in various stages of development and thereby concluded that the organisms had been leading a trophic existence. He calls attention to the inadequacy of the old "egg and germ" theory to account for the large numbers found. This matter will be discussed further in this paper.

The number of protozoa in soils varies widely with the nature of the soil. But there are very few soils that have not a fairly large number of cells belonging to numerous genera and species. No soil was examined in the state of New Jersey which did not contain some protozoa. The number of individuals present varies from a very few to many thousands per gram of soil. The dilution method of determining the number in soil is perhaps the most accurate method that can be used. Varying dilutions of the soil being studied, are made up and inoculated into suitable media such as soil extract or horse-dung infusion. These media are then examined microscopically for living protozoa daily up to about 30 days. It is obvious that a method which determines the number of cells in a culture solution *after* being inoculated for several days is inaccurate on account of the rapid multiplication of the organisms. Also certain species excyst after a few hours and some only after some days, hence it would be almost impossible to determine all the organisms present. By means of the dilution method the relative numbers of any species may be accurately determined although the procedure is time-consuming. Sherman (31) found some soils containing 10,000 protozoa per gram. Rahn (27) found an average of about 100 ciliates and from 1000 to 10,000 flagellates. Waksman (34) reports from 10 to 100 ciliates and 100 to 10,000 flagellates per gram of soil. In these estimates the numbers of rhizopods present in the soil are not given except very approximately. It is the authors' opinion that amoebae are very widely distributed in the soil. Since, as a rule, they excyst only after a considerable period, they are no doubt frequently overlooked. Wilson (37) substantiates this view when she refers to *naegleria* (*amoeba*) *gruberi* as the "common soil amoeba." Martin and Lewin (22) in England also found many amoebae in soils. Under ordinary conditions Waksman found no protozoa at a greater depth than 12 inches and showed that the greatest numbers are concentrated in the top 4 inches of the soil.

Regarding the presence of *living* protozoa in soil, much has been written. The results reported by various investigators are conflicting. This is to be expected, however, since the character of the soil, its moisture and organic content and its bacterial and protozoan life needs must vary in different parts of the world. In general, the English investigators incline to the view that protozoa in a trophic condition are constantly present in most soils. On the other hand, the American investigators' findings have demonstrated just the reverse. Whether the English workers drew their conclusions correctly from the results obtained has been discussed in the literature. Since the very thorough work of Koch (15, 16) and Waksman (34, 35) was carried out in New Jersey, their conclusions may be applied to New Jersey soils. Koch concludes protozoa do not exist in normal soils or even in soils where the moisture content is somewhat above normal. He refers here to field soils in particular. In greenhouse soils containing much organic matter and with high moisture content he found a few living protozoa. He thinks that protozoa may become active in soils whenever the moisture content rises considerably above the normal. Waksman found very few living protozoa in field soils and this investigator shows that both moisture and organic content are the principal limiting factors for the development of protozoa in the soil. Our work is entirely in accord with that of these two investigators; namely, that in ordinary soils there are very few living protozoa even though this same soil may contain thousands of cysts per gram. In the examination of some hundred fresh samples of soil, but two were found to contain living protozoa on the direct examination. In one case several *monas guttula* were observed in a heavy clay soil of the Penn series, and in the other case a few specimens each of *naegleria gruberi* and *colpoda cucullus* were demonstrated from a shaley loam soil of the same series. In water-logged soils and in samples obtained near ditches and ponds many species of *living* protozoa were present.

Purdy and Butterfield (25), with pure, bacteria-free cultures of protozoa, have recently shown that some of the common species of protozoa cannot exist in culture solutions containing organic matter, without the presence of living bacteria. They have proven that bacteria form the main food for protozoa and that the latter cannot subsist on organic matter, as was claimed by some investigators. In culture solutions the numbers of protozoa present always increased directly at the expense of the bacteria. Several investigators using impure cultures (i.e., cultures of protozoa mixed with bacteria), have shown that protozoa are bacteria eaters. Among these are Huntemüller (13) and Hoffman (12), who showed conclusively that certain water protozoa ingest typhoid bacteria. Among the species mentioned are two common soil flagellates, viz., *bodo ovatus* and *bodo saltans*. This is of interest, as Sherman (31) in his extended researches found that flagellates did not limit bacterial numbers in solution and soil. He found that the ciliates were capable of causing a large decrease in numbers of bacteria in culture solutions and in soil under proper conditions. Waksman's (35) results are in general in accord with Sherman's. It is thus seen that at least some species of protozoa use bacteria regularly as food, and it follows that the protozoa *living* in the soil also ingest bacteria. Since flagellates are the most common of all the protozoa and as Sherman found in general that they did not consume many bacteria, they are not perhaps of much consequence in the soil. However, this point needs to be more thoroughly investigated before any conclusions can be safely drawn. Goodey (10) shows that active protozoa exist in certain sewage-treated soils in England and may be a limiting factor in the fertility of those soils. This is undoubtedly true, and even in some of the wet, low-lying soils of New Jersey, the protozoa present may have a considerable detrimental influence upon the bacteria which are present. Russel and Huchinson (29, 30) claim that when soils are partially sterilized by heat or antiseptics the beneficial effect noted on the subsequent crop is a result of the destruction of an injurious biological factor (protozoa supposedly). Much controversy has arisen concerning this veiwpoint but it is probably the consensus of opinion of soil biologists that the well-known beneficial effect upon the soil is due to a number of other reasons. Under certain conditions it cannot be denied that protozoa might be able to decrease the fertility of a soil by destroying the beneficial soil bacteria.

ENUMERATION OF SPECIES OF PROTOZOA AND ALGAE IN NEW JERSEY SOILS

For the identification of these organisms, great care was taken to consult thoroughly the literature of protozoology to make certain of the correct nomenclature. In some cases this has been found to be very difficult because of the often imperfect descriptions and figures of the early investigators combined with the over-zealousness of others in making new genera and species when existing classifications were entirely adequate. It is not uncommon for a single organism to be described in the literature under five or six different names. In compiling the list below, the name of the investigator who named the species is always mentioned so that no doubt may be entertained as to the organism described. As the literature on the subject is somewhat scattered, some of the more useful works found for the identification of these organisms are given in the bibliography. Among them are Butschli (1), Kent (14), Calkins (2, 3), Döflein (6), Leidy (20), Stokes (33), Conn (4), Edmondson (7), Stein (32), Whipple (36) and Wolle (39, 40).

In the plates appended to this paper the more common soil protozoa and algae are roughly pictured. The drawings were originally made from the living and stained organisms themselves. In a few of the drawings, figures taken from the literature were used in connection with the original drawings.

The following species were identified.[1]

A list of the protozoa and algae found in New Jersey soils

(For drawings of the commoner forms see plates 1–4)

Class—Sarcodina
Subclass—Rhizopoda
- Hyalodiscus (amoeba) limax Duj. ++
- Hyalodiscus (amoeba) guttula Duj. ++
- Amoeba proteus Ehrbg. ++
- Amoeba radiosa Ehrbg. ++
- Naegleria (amoeba) gruberi Schardinger (Wilson) ++++
- Arcella vulgaris Ehrbg. +
- Difflugia pyriformis Perty +
- Difflugia globulosa Duj +
- Pamphagus mutabilis Bailey +
- Microgromia socialis Arch. +
- Diplophrys archeri Bark. +
- [2]Mastigamoeba sp (?) ++
- [2]Ciliophrys infusionum Cienk. +

Subclass—Heliozoa
- Actinophrys sol Ehrbg ++
- Vampyrella lateritia Fres. +
- Raphidiophrys sp. (?) +
- Clathrulina elegans Cienk. +

Class—Mastigophora
Subclass—Flagellidia
- Monas termo Ehrbg. (Martin) ++++
- Monas guttula Ehrbg. +++
- Monas vivipara Ehrbg ++
- Oikomonas mutabilis S K. (Kent) +++
- Cercemonas crassicauda Duj ++++
- Cercemonas longicauda Duj +++
- Pleuromonas jaculans Perty ++
- Physomonas elongata Stokes ++
- Monosiga ovata S. K. +
- Phyllomitis amylophagus (?) Klebs ++
- Bodo (heteromita) ovatus (caudatus) S. K. +++
- Bodo globosus Stein ++
- Bodo lens Müll. (S. K.) +
- Bodo sp (?). Resembles Conn No. 79 +
- Clostenema socialis (?) Stokes +
- Tetramitis variabilis Stokes +
- Hexamitis inflatus Duj. +
- Euglena viridis Ehrbg. ++
- Euglena acus Ehrbg ++
- Phacus pyrum Ehrbg. ++

---

[1] Relative abundance of species is indicated by plus (+) marks as follows: rare +; occasional ++; common +++; very common ++++ Identification doubtful (?).

[2] These two forms are often included in the Mastigophora.

Class—Mastigophora
Subclass—Flagellidia—*Continued*

Phacus longicaudus Ehrbg. +
Eutrepia viridis (?) Perty +
Trachelomonas volvocina Ehrbg. +
Cryptoglena pigra Ehrbg. ++
Astasia trichophora (?) Ehrbg. ++
Astasia sp. (?). Resembles Conn No. 73 +
Paranema trichophora Ehrbg. ++
Heteronema acus Ehrbg. ++
Petalomonas mediocanellata (?) Stein +
Mallomonas sp. (?). Resembles Edm. No. 85 +
Crytomonas ovata Ehrbg. ++
Chilomonas paramecium Ehrbg. (Stokes) ++
Chilomonas sp. (?). Resembles Conn No. 95 +
Chlamydomonas sp. (?) ++

Class—Infusoria
Subclass—Ciliata

Holophrya sp. (?). Resembles Conn No. 120 +++
Holophyra sp. (?). Resembles Conn No. 121 ++
Urotricha sp. (?). Resembles Edm. No. 91 ++
Urotricha (balantozoon) agile Stokes ++
Enchelys farcimen Ehrbg. ++++
Enchelys sp. (?). Resembles Conn No. 124 + +
Prorodon teres (?) Ehrbg. ++
Prorodon armatus (?) C. & L. ++
Mesodinium sp. (?) +
Lionotus fasciola Ehrbg. ++
Loxophyllum flexilis Stokes ++
Dileptus gigas C. & L. ++
Dileptus sp. (?). Resembles Conn No. 160 ++
Chilidon cucullulus Müll ++
Chilidon megalotrochae Stokes +++
Trichoda pura Ehrbg. (Edm. No. 118) ++
Glaucoma scintillans Ehrbg +++
Colpidium striatum Stokes +++
Colpidium colpoda (?) Stein +++
Uronema marina Duj ++
Colpoda (tillina) saprophila Stokes +++
Colpoda cucullus Stein ++++
Colpoda campyla Stokes +++
Colpoda helia Stokes ++
Colpoda flavicans (?) Stokes +
Parambecium trichium Stokes +
Paramoecium caudatum Ehrbg +
Pleuronema chrysalis Ehrbg ++
Pleuronema sp. (?). Resembles Conn No. 211 ++
Cyclidium glaucoma Ehrbg. +++
Lembus pusillus (?) Quenn. (Calk.) +
Blepharisma (apgaria) ovata (?) Stokes +
Metopus sigmoides C. & L. ++
Metopides acuminata (?) Stokes +

Class—Infusoria
Subclass—Ciliata—*Continued*

- Strombidium sp. (?). Resembles Conn No. 229 +
- Halteria grandinella O. F. Müll. +++
- Stichotrica secunda Perty +
- Uroleptus dispar (?) Stokes ++
- Uroleptus musculus (?) Ehrbg. +
- Oxytricha pellionella Müll. +++
- Oxytricha bifaria Stokes ++
- Stylonichia mytilis Ehrbg. +++
- Stylonichia pustulata Ehrbg ++
- Euplotes charon Müll. (Ehrbg.) ++
- Euplotes carinata Stokes +
- Aspidisca costata Duj. ++
- Vorticella striata Duj. ++++
- Vorticella citrina Ehrbg. +++
- Vorticella putrina Müll. ++
- Vorticella microstoma Ehrbg. +++
- Vorticella globularia Müll. ++

Soil algae and diatoms

- Protococcus viridis Ag. +++
- Protococcus sp. (?) +++
- Botryococcus sp. (?) +
- Tetraspora sp. (?) ++
- Chroöcoccus cohoerens (?) Naeg. ++
- Nostoc sp. (?) ++
- Closterium sp. (?) ++
- Cosmarium sp. (?) +
- Coelosphaerium sp. (?) +
- Ulothrix sp. (?) ++
- Spirogyra sp. (?) +

- Diatoma vulgare ++
- Navicula rhyncocephara (?) ++
- Stauroneis sp (?) +
- Cymbella sp. (?) ++
- Nitzchia sp. (?) ++
- Synedra sp. (?). +

Discussion

Contained in this list are 13 species of rhizopods, 4 heliozoa, 34 flagellates, 51 ciliates; and in addition to the protozoa are 11 algae and 6 diatoms. It is thus seen that from a species standpoint, the cilates are most numerous, followed in order by the flagellates, rhizopods and heliozoa. In point of actual numbers in the soil, however, flagellates easily rank first, followed by the ciliates and rhizopods. The larger size of the ciliates tends to diminish the advantage which the flagellates have from the standpoint of numbers. Comparing these results with those obtained by Kofoid (17) in his study of the plankton of the Illinois, Spoon and Quiver rivers we find fair agreement.

In the Illinois river he found 30 rhizopods, 4 heliozoa, 62 mastigophora, 55 ciliata and 5 suctoria. In the Spoon River he found 17 rhizopods, no heliozoa, 38 mastigophora, 16 ciliata and 2 suctoria, while in the Quiver River the only organisms found were 13 rhizopods and 5 mastigophora. With respect to species relationship, then, the soil microfauna is not unlike that of fresh water. The main points of difference are in the absence from, or very limited presence in soil of such forms as the suctoria, rotifera and crustacea. It appears that they cannot withstand the desiccation and other unfavorable conditions which exist in soil.

Compiling those species which have been classed as abundant or common in New Jersey soils we find that there are 17 ciliates, 6 flagellates, 1 rhizopod and 1 alga. Seven of the cilates, however, belong to two genera, namely *colpoda* and *vorticella.* As regards abundant species the ciliates are again first, with flagellates second. Only 6 species are classed as "very abundant." These are *naegleria gruberi*, *monas termo*, *cercemonas crassicauda*, *enchelys farcimen*, *colpoda cucullus* and *vorticella striata.* Several other genera, such as the *bodos*, *monas*, *halteria*, *colpidium* and *stylonichia* were almost as common.

Of course, a considerable number of organisms were encountered which could not be even tentatively identified. Several fairly common ciliates and flagellates fall into this class. Some are undoubtedly new species and some may even have to be given new generic names. The soil protozoa would furnish the systematist with rich material for investigational work.

The algae and diatoms were not thoroughly studied but are mentioned in this connection mainly because of their close relationship. The algae belong mainly to the coccus forms although a few filamentous genera were found. In order to cultivate the algae in soil, the latter was mixed with quartz sand in a flask, saturated with a weak soil extract and left in the sunlight for several months. Under these conditions the algae present will form a mat on the surface of the soil-sand mixture. Soil algae may be of importance in soil fertility by furnishing energy material for the growth of certain beneficial soil bacteria such as the *azotobacter*. By virtue of the ability of algae to manufacture chlorophyll in the presence of sunlight and of the ability of *azotobacter* to fix atmospheric nitrogen, a mutual beneficial association of these two organisms might result. Beijerinck, in Holland, has shown that such an association actually does take place in the soil; the algae furnishing the necessary carbohydrate and the bacteria, the nitrogenous matter. D. A. Coleman, in unpublished data, has found that *nostoc* incubated in flasks in the sunlight with several mixtures of *azotobacter* does not furnish appreciable energy-material for the fixation of atmospheric nitrogen. Of course, in the field conditions may be such that appreciable fixation takes place.

To throw some light upon the question of the similarity of the microfauna of the soil and that of neighboring ditches, pools and ponds, many samples taken from the latter sources were studied. In general, the fauna was much more varied in character than that of the soil. Rotifers, crustaceans, diatoms, suctoria, sponges and certain colonial protozoa were to be found in the fresh-water ponds and streams and not in the soil. In both soil and water, nematodes and larvae of insects were often encountered. However, nearly all species which were found in soil in New Jersey have been also identified from fresh-water sources either in New York or in New Jersey. Their relative abundance, however, is not necessarily the same in soil and water; in fact it is to be noted that some very common plankton organisms were never found in the soil, and *vice versa*, some of the common soil protozoa are not often found in fresh-water. The work of Stokes (33) in New Jersey and other states, Conn (4) in Connecticut, Edmondson (7) in Iowa, and Kofoid (17) in Illinois, on the fresh-water species of protozoa, shows that the same organisms are present in nearly every locality but not in the same relative abundance. This apparently is true also in the case of soils.

It was plainly noticeable that many of the terrestrial protozoa and algae often differed materially in size, color and in some other characteristics from the same organism taken from fresh or slightly brackish water. Sometimes a sheath or membrane was absent, a green form would be colorless and so-called "social species" would be "solitary" in the soil. That environment has brought about these changes seems evident. This observation also materially strengthens the conjecture that there is a fairly definite soil microfauna and that the protozoa lead a trophic existence whenever soil moisture becomes excessive. Such a condition takes place after heavy rains, floods and in the spring when frozen soil is thawing out.

The characteristic protozoa of the soil, then, are those simple, small, hardy forms which are capable by means of rapid encystation or otherwise, to withstand successfully extremes of heat and cold, desiccation, aeration and such other inhibiting factors as are natural to their home in the soil.

DISTRIBUTION OF PROTOZOA IN DIFFERENT SOILS

Thirty-five soils were collected for a study of the distribution of protozoa in different soils. All but three of these were typical New Jersey soils and represented nearly every county in the state. The work of identification of species was naturally incomplete, for the reason that sufficient time could not be given to any one soil sample to insure getting every organism there. Two media were used—soil extract and horse-dung. One-hundred-gram portions of each soil were used in each of the two media. In this connection it should be stated that of five media tested out for soil protozoa, namely, blood-meal extract, 1 per cent beef broth, hay infusion, soil extract (heated in an autoclave), and horse-dung infusion, the latter two were found to be best suited to our work. The main difficulty with the others was that bacterial growth was so heavy as to prevent the development of the protozoa; the solutions often becoming putrescent. The flasks were examined daily for two weeks,

TABLE 1

Group relationship and distribution of protozoa in soils

SOILS	FLAGELLIDIA	CILIATA	RHIZOPODA	HELIOZOA	DIATOMS	ALGAE	TOTAL SPECIES OF PROTOZOA*
1A. Middlesex County	8	8	1	0	1	1	17
3A. Middlesex County	8	16	3	1	0	0	28
5A. Middlesex County	4	10	6	0	0	1	20
6A. Middlesex County	6	10	2	0	0	0	18
7A. Middlesex County	3	5	1	0	2	1	9
8A. Middlesex County	11	13	0	1	0	3	25
Sussex County	3	7	2	0	0	0	12
Burlington County	6	2	2	0	0	0	10
Monmouth County	9	7	2	1	0	0	19
Cumberland County	7	6	0	0	0	1	13
Atlantic County	6	11	3	3	1	2	23
Cape May County	1	1	0	0	0	0	2
Morris County	3	2	0	0	0	0	6
Hunterdon County	2	1	1	0	0	0	4
Warren County	6	5	0	0	0	0	11
Union County	1	2	1	0	0	0	4
Ocean County	1	4	0	0	0	0	5
Bergen County	6	5	0	0	0	0	11
Somerset County	6	5	1	0	0	0	12
Mercer County	7	1	1	0	1	0	9
Essex County	2	3	1	0	0	1	6
Camden County	4	2	0	0	1	0	6
Passaic County	10	5	3	1	0	2	19
1. Middlesex County	2	5	1	0	0	0	8
2. Middlesex County	1	0	1	0	0	0	2
3. Middlesex County	2	2	0	1	0	0	5
4. Middlesex County	2	5	1	0	1	1	8
5. Middlesex County	4	13	1	1	0	2	19
6. Middlesex County	8	5	1	0	0	0	14
7. Middlesex County	4	7	0	0	0	0	11
8. Middlesex County	8	8	2	0	1	0	18
9. Middlesex County	2	4	0	0	0	0	6
10. Middlesex County	4	3	1	1	3	0	9
Colorado (normal)	3	3	0	0	0	0	6
Colorado (nitre spot)	1	3	0	0	0	0	4
Florida (sandy)	2	1	2	0	0	0	5
Totals (New Jersey soils only)*	157	184	38	10	11	15	389
Average number of species per soil (nearest integer)	5	6	1	Less than 1	Less than 1	Less than 1	12

* Algae and diatoms not included in totals.

then semi-weekly for two weeks more. This series of samples was examined for evidence of living protozoa but with negative results. In some cases, however, the samples had partly dried out by the time they arrived at the laboratory, and had there been any free living organisms in the fresh soil they might have encysted again.

Discussion

The results given in table 1 indicate that protozoa are present in some form or other in all the soils examined. The largest number of species identified from any one soil was 28, this large number being obtained from a fertile but heavy clay of the Penn series. The smallest number of species found in any soil was 2, in both an acid virgin forest soil from Middlesex County and a coarse sandy soil from Cape May County. In general it seemed to make but little difference where the samples of soil were obtained —a fair number of organisms were always secured. Certain species, however, appeared only in certain soils, for example ***dileptus gigas*** and ***uroleptus musculus*** were found only in Sussex County soils, ***euglena acus***, in a rich black forest soil of Middlesex County, and ***difflugia pyriformis*** as well as ***vampyrella lateritia*** were identified from but one locality in Middlesex County. Similar observations have been made regarding the distribution of certain fresh-water organisms. In certain pools one may always find certain species, yet again he can search for days in other localities and not encounter them. This unique circumstance regarding distribution of species in fresh water apparently holds also in the case of the soil microfauna.

RELATION OF PROTOZOA TO SOIL TYPE, SOIL BACTERIA AND MOLDS

This relation was studied only in a very incomplete manner, but as some facts of interest were indicated by the work, it is here reported. It was generally observed that the soils apparently in a high state of fertility practically always showed the presence of more species and numbers of organisms than did the less fertile soils. To test out this observation further the following data are offered. More direct evidence is required, however, before the question can be definitely decided.

The data contained in table 2 show that of the soils studied, those in a high state of fertility harbored the greatest number of protozoa as well as the greatest number of species. The same is also true regarding the number of bacteria present. Molds do not apparently follow this general rule for nearly as many were noted in an acid soil under sod as in a virgin forest soil. The acid soil had received large amounts of ammonium sulfate annually for some years previous. The forest soil was also acid. The other soils tested were either only very slightly acid or neutral in reaction. Field conditions seem to show that the same relation holds between numbers of protozoa and bacteria in the soil as well as in the culture flask. In other words, where there are the greatest numbers of bacteria, there also do we find the greatest number of protozoan cells and species. Such a statement must be broadly interpreted, as it is reasonable to suppose that there are many instances where this relation does not hold. For example, very wet soils may be very low in numbers of bacteria but still contain many protozoa, also where bacterial putrefaction is taking place and large numbers of bacteria are present, protozoa may be almost entirely inhibited. Such a condition is often observed in culture flasks containing blood meal or beef extract. In many cases, however, a high bacteria count and high protozoa count (or at least a large number of species) go together. This might be explained by supposing that during the periods of their activity in the soil the protozoa find great quantities of food and are thus able to propagate rapidly while conditions are favorable. As the soil dries out they go into encystment again. This seems to be the logical explanation of the facts, especially since Goodey (10) and others have shown that encystation and excystation can take place in a few hours under suitable conditions.

TABLE 2

SOURCE OF SOIL SAMPLES	NUMBER OF PROTOZOA PER GRAM*	NUMBER OF BACTERIA PER GRAM	NUMBER OF MOLDS PER GRAM	NUMBER OF SPECIES OF PROTOZOA FOUND IN SAMPLE
Cultivated rich garden soil	3500	5,300,000	125,000	26
Alfalfa sod (rich)	4500	5,000,000	90,000	23
Cultivated orchard (poor) .	1000	4,850,000	85,000	9
Very acid soil (sod)	Very few	960,000	138,000	4
Virgin forest	55	1,500,000	110,000	3

* The data in this column were obtained from Dr. S A Waksman

IS THERE A TRUE SOIL MICROFAUNA?

If we consider this question in the light that the soil protozoa are distinct and different species altogether from the fresh-water forms, then we must say that there is none. But, on the other hand, if we mean by "true soil microfauna," those organisms which are practically always present in the soil in considerable numbers and which use the soil as a medium in which to live and carry on their life processes, we must say that there is. It has been observed that just because an organism may be extremely abundant in fresh water it does not necessarily follow that it is also common in soil. The converse of this statement also is true. A striking example of this nature is in the case of *paramoecium caudatum*. This large easily identified protozoan is extremely abundant in fresh water, yet it is but rarely encountered in New Jersey soils. *Naegleria gruberi*, the commonest soil rhizopod found, is encountered only occasionally in plankton studies. In general, we may say that a considerable number of species of protozoa are present in fairly large numbers in most soils. Certain species of algae also seem to be common in soil; the most common form being the *protococcus.* As *rotifera* and the more complex plankton organisms, with the exception of nematodes, do not occur except very rarely in soils, they have little significance in this consideration.

SUMMARY

1. Protozoa were found in all the soils examined; the number of species identified from any one soil varying from 2 to 28. Poor sandy acid or forest soils contained the least number of species, while fertile soils, rich in organic matter or with high water-holding capacity, contained the greatest number.

2. Of the 104 species identified from New Jersey soils, 51 were ciliates, 35 were flagellates, 14 were rhizopods and 4 were heliozoa. In addition to the protozoa 10 genera of algae and 6 of diatoms were identified. Nematodes were common, but rotifers or crustaceans were not encountered.

3. Of the species classed as being abundant in the soil, 17 were ciliates, 6 were flagellates and 1 a rhizopod. The ciliates outnumber the flagellates in the number of species identified but *not* in actual numbers of organisms in the soil. In point of numbers the rhizopods and heliozoa rank third and fourth, respectively. No suctoria were encountered.

4. It is believed that in normal[3] New Jersey soils protozoa exist mainly in a non-trophic state. Only two soils out of some hundred samples taken all over the state showed the presence of living protozoa on direct examination. In soils which are saturated with water for several hours, or in standing soil-water, field ditches and plough-furrows, limited numbers of protozoa may usually be found. Since their activity is very limited, especially at low temperatures, they can have but little importance from the standpoint of soil fertility under New Jersey conditions.

5. The soil microfauna consists principally of those small, simple and hardy protozoa which are able to withstand by means of encystation or otherwise, such extremes of heat and cold, desiccation, aeration, etc., as are natural to their life in the soil.

6. Practically all the species which were identified from the soil have also been found in the fresh-water lakes, ponds, pools and streams of the state, but *not* in the same relative abundance. Some species which are abundant in soil are rarely encountered in fresh water, and *vice versa,* several of the most common plankton organisms in the state are but rarely found in soil.

7. A new theory to replace the old "egg and germ" theory of distribution of protozoa in soil is put forth as follows:

Some protozoa undoubtedly are spread about by rain, winds, animals and other natural forces. It is not believed that these means are sufficient to account for the large number of organisms of many species found in all soils. Protozoa become active in soil whenever there is excessive moisture present for a period of several hours. Such conditions are common during prolonged rains especially in the rainy seasons, during winter and spring thaws, in soils kept moist from seepage and in poorly drained lands. Excystation takes place followed by a feeding period and consequent growth, then reproduction takes place. As conditions become unfavorable again, encystation begins or death occurs. Since all these processes may take place in the course of a few hours (but usually requiring 24 hours at least for appreciable reproduction to take place), the whole life cycle of many protozoa may be completed in the soil.

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[3] Soils not containing moisture much in excess of the physical optimum.

In support of this contention it may be stated that there is apparently a fairly definite soil fauna which differs in several respects from the surrounding fresh-water fauna. Rich, fertile soils contain greater numbers of both bacteria and protozoa than poor or very acid soils. Since protozoa use the bacteria as food it seems reasonable that they would multiply more readily in places where there is plenty of food. Protozoa may be generally found in field ditches, plow furrows, in soil where there is standing water, and in soils wet from seepage, showing that they may lead a trophic existence in soil if conditions are suitable. It is absolutely unreasonable to conceive of the elements and other natural forces as being accountable for 5000 protozoa in practically every gram of soil in the state.

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PLATE 1

RHIZOPODA

1. *Hyalodiocus limax* Duj 30–80 μ (45), occasional; simple, hyaline with single pseudopodium.

2a. *Hyalodiscus (amoeba) guttula* Duj. 10–25 μ (20), occasional; a very minute rhizopod; usually club-shaped; endoplasm clear with few large granules.

2b. Same, with pseudopodium extended

3. *Amoeba proteus* Ehrbg 70–250 μ (150); occasional; a large many-lobed organism.

4a. *Naegleria (amoeba) gruberi,* Schardinger (Wilson). 15–40 μ (25); trophozoite; very abundant; drawing shows nuclear change taking place

4b. Same; one pseudopod extended while the other is retracted.

4c. Same, flagellated stage 15–30 μ (20), this stage found only occasionally in cultures but often on exposed microscopic slides

5a. *Amoeba radiosa* Ehrbg. Body 15–35 μ (18); occasional, this is usual form

5b. Same, radial spikes having changed into pseudopodia.

6. *Arcella vulgaris* Ehrbg 50–90 μ (70); occasional; top-view showing delicate finger-like pseudopodia beneath the shell.

7. *Mastigamoeba* F E Sch sp? 15-35 μ (30); occasional; short, blunt, unbranched pseudopodia. Usually classed with the *Mastigophora* on account of the flagellum A variable genus

8. *Difflugia pyriformis* Perty 40-200 μ (90), occasional, slender, delicate pseudopodia extending from beneath the silicious shell

9. *Microgromia socialis* Arch Rare Individuals vary in size from 8–14 μ (12); a colonial form, pseudopodia not anastomosing in some specimens

10 *Actinophrys sol* Ehrbg 50–100 μ (70), occasional, specimen much vacuolated; one of the commonest *Heliozoa* found in the soil

11. *Diplophrys archeri* Bark. 20–35 μ (25); rare, showing several contractile vacuoles and oil globules

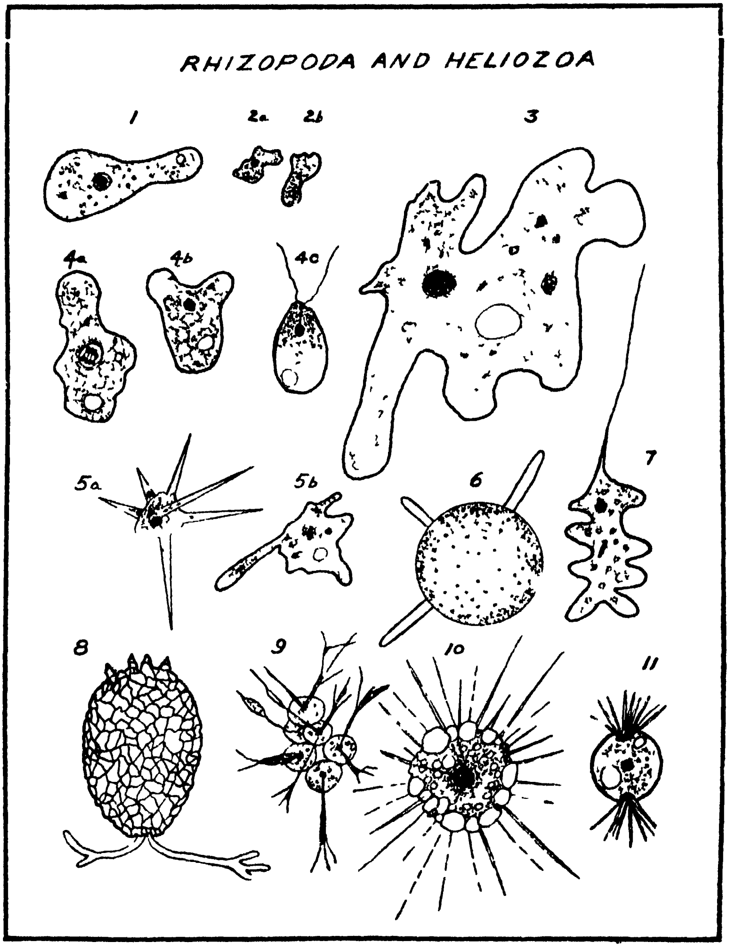
RHIZOPODA AND HELIOZOA
1
2a
2b
3
4a
4b
4c
5a
5b
6
7
8
9
10
11

PLATE 2

Mastigophora (Flagellates)

1. *Monas termo* Ehrbg. (Martin); 5–10 μ (7); very abundant; usual shape globular to ovoid.

1a. Same, ovoid form of this species; body somewhat changeable in shape.

1b. Same; showing this organism with 2 flagella ready to divide.

2. *Monas guttula* Ehrbg 7–15 μ (12); abundant; posterior nucleus and posterior vacuole.

2a. Same; semi-amoeboid form showing 2 dissimilar flagella.

3. *Monas vivipara* Ehrbg. (Stein); 12–25 μ (17); sometimes attached at base; occasional.

3a. Same; showing a common amoeboid form; nucleus visible with reagents.

4. *Oikomonas mutabilis* S. K. (Kent); 7–15 μ (10); abundant; sometimes attached at base.

4a. Same; showing a specimen with distorted body and single flagellum.

5. *Cercemonas crassicauda* Duj. 10–25 μ (18), very abundant; shape variable.

5a. Same; semi-amoeboid form, flagella sometimes appear as simply body prolongations.

6. *Physomonas elongata* Stokes. 7–15 μ (9); occasional; body usually twice as long as broad.

6a. Same; showing changeable shape; sometimes attached by a posterior pedicel.

7. *Pleuromonas jaculans* Perty. 5–11 μ (8); occasional; kidney-shaped to spherical.

7a. Same, showing a more spherical form attached by posterior flagella, nucleus, anterior.

8. *Mallomonas,* Perty, sp. Edm ? 12–28 μ (18); rare, body covered with rigid hairs or setae.

9. *Phyllomitis amylophagus* Klebs (?), Conn. 10–22 μ (16); occasional; one flagellum often trailing.

9a. Same; a smaller form with a more flexible body.

10. *Bodo ovatus* Duj. Average size 14 μ x 8 μ, abundant; one flagellum trailing while the other is used as a propulsive whip anteriorly.

11. *Bodo globosus* Stein 8–12 μ (9), abundant, almost spherical in form.

12. *Bodo,* Ehrbg. sp (?), *Heteromita* (Stokes). 10–14 μ (11), occasional; an oblique, longitudinally ridged specimen.

13. *Cryptomonas ovata* Ehrbg. 20–250 μ (30); occasional, specimen shows green chlorophyll bands encircling the body.

14. *Chlamydomonas,* Ehrbg sp. (?). 12–30 μ (20), occasional, endoplasm green, single eyespot.

15. *Trachelomonas volvocina* Ehrbg 25–50 μ (35), occasional; color usually brown; sometimes without neck-like projection.

16. *Cryptoglena pigra* Ehrbg. 10–20 μ (13), occasional; 2 pigment bands and single eyespot.

17. *Chilomonas paramecium* Ehrbg (Stokes), 20–40 μ (28); occasional, vacuole located anteriorally; dark colored corpuscles scattered in the endoplasm.

18. *Monosiga ovata* S K. 10–15 μ (12), rare, solitary, often attached to debris.

19. *Hexamitus inflatus* Duj 12–22 μ (15): rare; 4 vibratile anterior and 2 posterior trailing flagella

20. *Phacus pyrum* Ehrbg 15–50 μ (25); occasional; color greenish; eyespot; body obliquely grooved.

21. *Euglena viridis* Ehrbg 18–60 μ (30); occasional; body usually green; red eyespot; body shape variable, organism senstive to stimuli.

22. *Paranema trichophora* Ehrbg. 50–120 μ (90), occasional; body very flexible; nucleus central

23. *Heteronema acus* Ehrbg (Stein) 35–80 μ (50); occasional; body plastic; longer flagellum trailing.

24. *Astasia trichophora* Ehrbg. (?) 30–60 μ (45), occasional; body plastic; endoplasm is transparent, no eyespot present.

24a. Same, showing a commonly observed distortion

25 *Tetramitus variabilis* Stokes. 10–20 μ (16), rare, 4 subequal flagella, body obovate plastic and variable in shape.

26 *Euglena acus Ehrbg.* 40–100 μ (70), occasional; body very elongate, greenish in color; posterior end pointed, nucleus central; eyespot present.

PROTOZOAN FAUNA OF NEW JERSEY SOILS
C R FELLERS AND F E ALLISON

PLATE 2

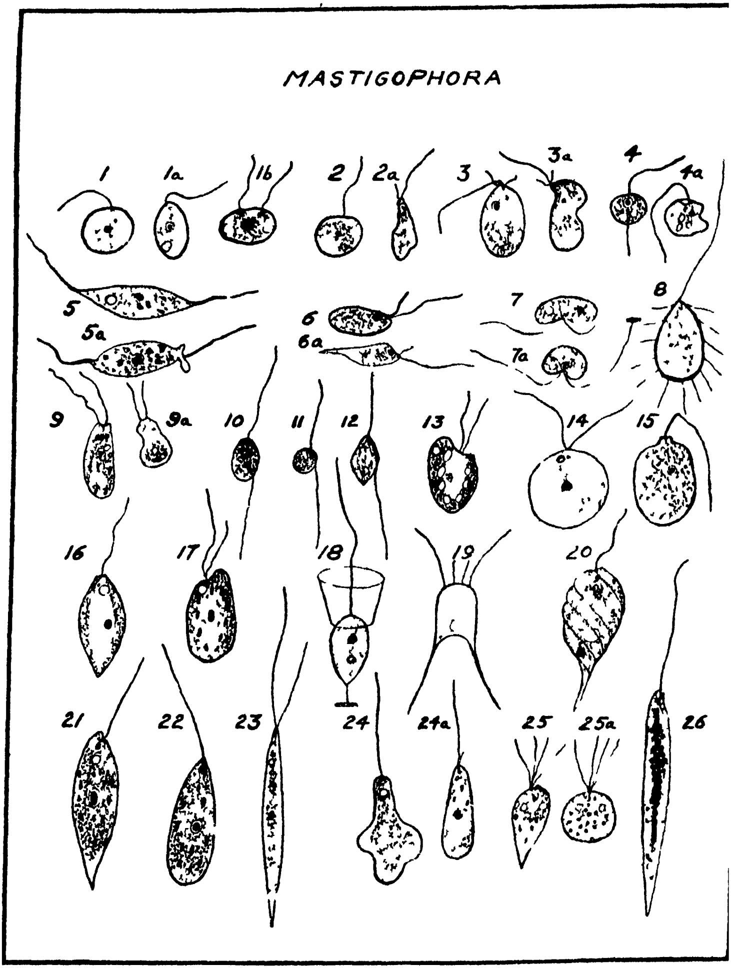

PLATE 3

CILIATA

1. *Holophrya*, sp. (?) Conn. 30–50 μ (38); a variable genus of many species several of which are common in the soil.

2. *Urotricha*, C. & L. sp. Edm. fig. no. 91. 35–50 μ (40); occasional; shows posterior springing hair and lip-like extensions of apical oral aperture.

3. *Enchelys farcimen* Ehrbg. 30–60 μ (40); very abundant; this is one of several similar organisms referred to this genus.

4. *Lionotus fasciola* Ehrbg. 90–120 μ (110); occasional; 2 spherical nuclei; oral aperture located ventrally but usually not visible.

5. *Prorodon teres* (?), Ehrbg. 60–150 μ (75); occasional; oral opening terminal; nucleus central; the usually described species is larger than this one.

6. *Dileptus gigas* C. & L. 200–700 μ (400); occasional; body elongate with narrow, flexible neck-like extension anteriorally; nucleus moniliform; contractile vacuoles numerous.

7. *Chilodon cucullulus* Müll. 70–150 μ (110); occasional; oral aperture located in central part of body, contractile vesicles numerous.

8. *Trichoda pura* Ehrbg 30–45 μ (35); occasional; endoplasm very transparent showing central oval nucleus, movements very rapid.

9. *Glaucoma scintillans* Ehrbg. 40–100 μ (55); common; oral aperture near anterior extremity with small vibrating membrane, movements very rapid

10 *Loxophyllum flexilis* Stokes 70–150 μ (115); rare; nucleus moniliform, body anteriorly pointed.

11. *Uronema marina* Duj. 22–40 μ (30); occasional, body ovate with a single posterior seta; oral aperture ventral with small membrane.

12. *Colpidium striatum* Stokes 45–75 μ (60); abundant; nucleus central; a posterior contractile vacuole; pharynx ventral with small undulating membrane

13. *Colpoda cucullus* Stein 30–50 μ (35); extremely abundant, nucleus central, contractile vacuole posteriorly located, distinct groove ventrally.

14 *Colpoda saprophila* Stokes 25–40 μ (30); abundant; ventral border anteriorly notched; recurved pharnyx very conspicuous, wide ventral groove. (This species is designated as "*Tillina saprophila*" by Stokes)

15. *Metopus sigmoides* Clap & Lach. 90–160 μ (120); occasional, body very contractile; contractile vacuole terminal; several long terminal setae.

16. *Paramoecium trichium* Stokes. 65–100 μ (85), rare, broad oral groove; double contractile vacuole at anterior extremity, nucleus oval and centrally located.

17 *Blepharisma* (*Apgaria*) *ovata* (?) Stokes 80–150 μ (100); rare, body much flattened; anterior end acute, membrane covering lower part of peristome sometimes visible.

18 *Cyclidium glaucoma* Ehrbg 18–30 μ (22); very abundant, body covered with long rigid cilia, hood-like membrane covers oral aperture; movements are darting and eccentric in nature.

19. *Pleuronema chrysalis* Ehrbg (Stein). 30–70 μ (45), occasional, body curved; a prominent undulating membrane extends from large the furrow-like peristome.

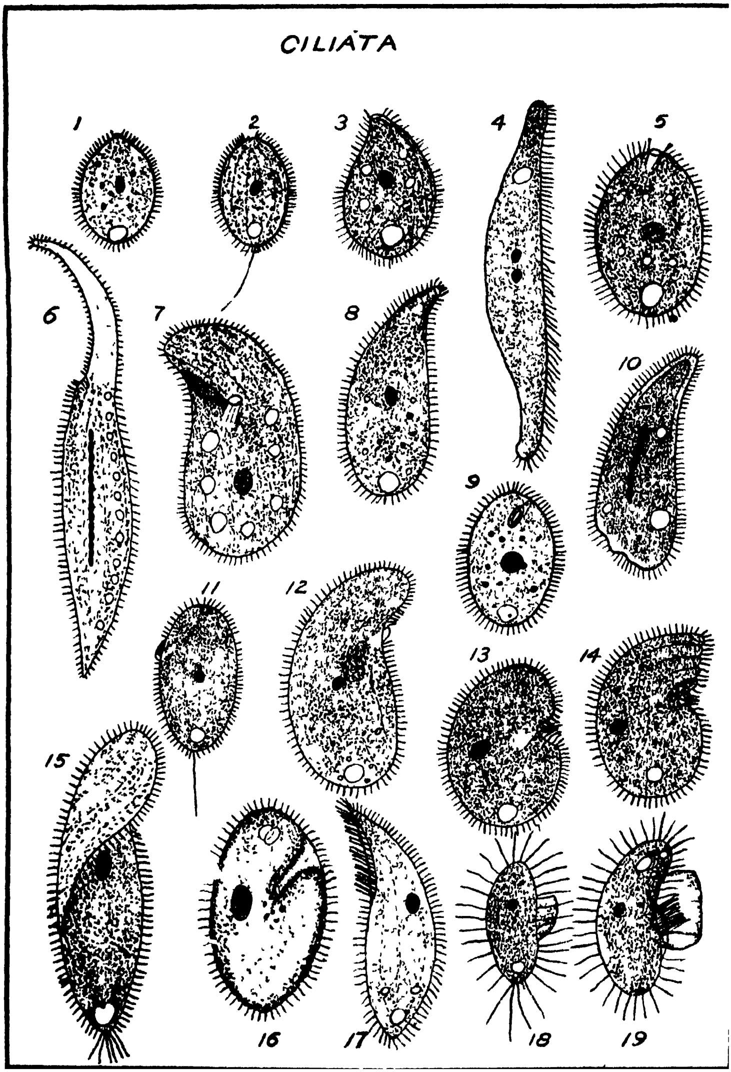
CILIATA
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PLATE 4

CILIATA (CONTINUED)

1. *Halteria grandinella* O. F. Müll. 25–35 μ (30); common; setae fine and long; movements springing and erratic.

2. *Strombidium*, Clap. & Lach. sp. Conn (?). 30–60 μ (40); rare; like *halteria* but has no bristles; movements very rapid and erratic.

3. *Oxytrichia pelionella* Müll. 70–100 μ (80); common; 2 oval nuclei; 2 anterior and 5 anal styles.

4. *Uroleptus dispar* (?) Stokes 125–200 μ (150); occasional; body elongate, oblanceolate and elastic; double ovate nucleus; 3 frontal and no posterior styles.

5. *Stylonichia mytilis* Ehrbg. 100–200 μ (135); common; (ventral view); 8 frontal, 5 ventral and 5 anal styles and 3 posteriorly placed setae.

6. *Euplotes charon* Müll. Ehrbg. 70–100 μ (80), occasional; (dorsal view); 7 frontal and 3 ventral styles, nucleus ribbon-like.

7. *Aspidisca costata* Duj. 30–50 μ (35); occasional, nucleus curved and ribbon-like.

8. *Vorticella striata* Duj. 35–45 μ (40); common in soil as are many other species of vorticella; striae prominent.

SOIL ALGAE AND DIATOMS

9. *Protococcus viridis* Ag. 3–20 μ; common; color green to red; cells single or in small clusters

10 *Closterium* sp. (?). 75 μ; occasional, color green.

11. *Nostoc* sp. (?) Cell diam. 4–6 μ; diam. of heterocysts and spores 8–10 μ; occasional; filaments bluish green in color and enclosed in a gelatinous sheath.

12. *Ulothrix*, sp (?) Cell diam 6–10 μ; rare, filaments simple and articulate; cell contents green and cell membrane thin.

13. *Botryococcus* sp. (?) 7–14 μ diam ; occasional; cells oval to spherical with thin confluent tegument; masses of cells densely packed forming a botryoid; color light green.

14. *Tetraspora* sp (?) Cell diam. 4–10 μ; occasional, cells angular to spherical; usually arranged in tetrads, gelatinous sheath sometimes present.

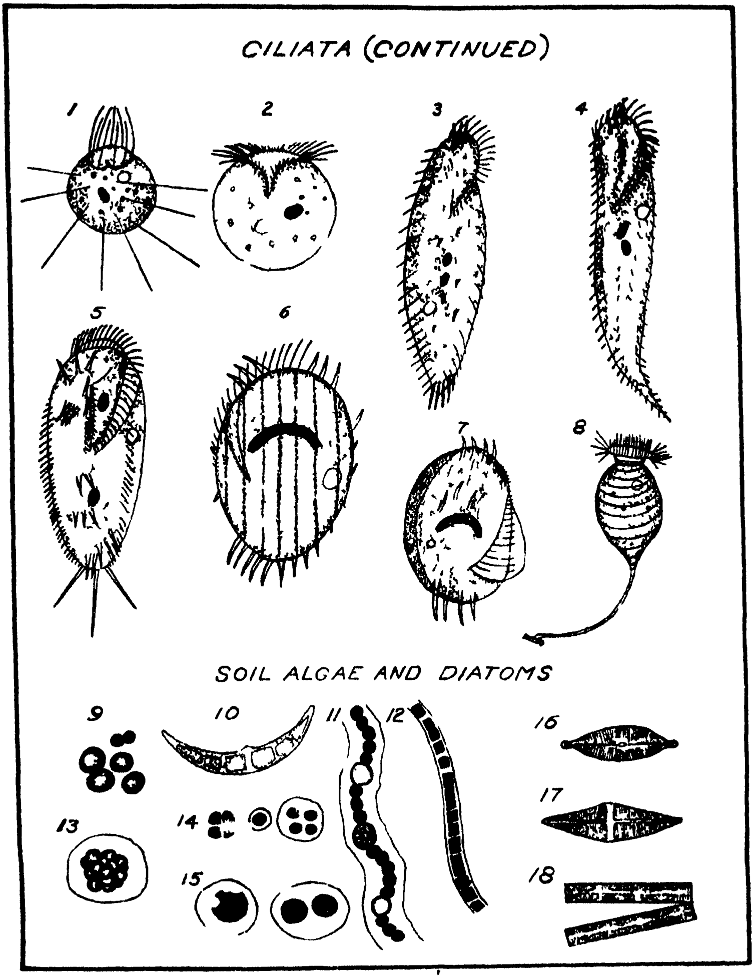
15. *Chroococcus cohoerens* (?) Naeg , 3–6 μ diam , occasional; solitary or in small groups; gelatinous sheath usually present.

16 *Navicula rhyncocephara* (?) 40 μ, occasional, valves symmetrical and boat shaped; raphé straight with conspicuous medial nodule.

17. *Stauroneis*, sp. (?) 45 μ; occasional, valves symmetrical; there is a clear area along the raphé which also extends up transversely from the medial nodule giving the appearance of a cross.

18. *Diatoma vulgare* (girdle view); 45 μ; rare; frustules attached by their angles forming zigzag chains; values elliptical-lanceolate and lined with fine striae; there is a longitudinal pseudo-raphé.

PLATE 4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SOIL ALGAE AND DIATOMS
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THE REACTION OF THE SOIL AS INFLUENCED BY THE DECOMPOSITION OF GREEN MANURES[1]

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INTRODUCTION

A common idea associated with the practice of green manuring is that the soil tends to grow more acid. This idea is a result, perhaps, of the ancient and still existing controversy relative to the existence of the so-called humic acids, and to a belief that these acids result from a decomposition of organic residues from green manures or other sources.

Temple (14) has raised the question as to what the effect of green manures is on soil acidity. From his work on nitrification after the addition of large amounts of organic acids, he concludes that injury due to the accumulation of organic acids in arable soils is very doubtful.

Hall (6) cited work at Rothamsted to show that the per cent of calcium in the first 9 inches of soil decreased where an annual application of 14 tons of manure per acre had been made.

Bear and Salter (2) showed that the lime requirement of manure plats became less than that of the check plats.

Hill (8) cited work of Laurent indicating that the harmful effects of green manure result from an accumulation of organic acids.

Ames and Schollenberger (1) have shown by pot experiments, conducted with soil taken from an uncultivated field, that the application of legumes and non-legumes in either dry or green state, has decreased the lime requirement as indicated by the Hopkins method. The dry application was more effective than the green.

White (15) found that when green manures and soil were kept in jars the lime requirements were less than with the soil alone at the end of the first fortnight, and more at the end of the second fortnight.

This brief review of the literature serves to indicate that the quantitative evidence for or against this question is very limited.

At the Rhode Island station there are plats, which, by their similarity of treatment in the past, offer opportunity for such a study and it is the purpose of this paper to present such information as has been secured.

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[1] Contribution 265 from the Agricultural Experiment Station of the Rhode Island State College.

HISTORY OF THE FIELD EXPERIMENT

On an acre of land, corn has been grown continuously since 1894 with uniform applications of only chemical manures as a source of plant-food. After a few years the plan of the experiment was modified so that a comparison between a legume and a non-legume, as a means of maintaining the soil humus, might be made. At the time of the last cultivation of the corn, each season, one-quarter was for years usually sown with crimson clover, while another quarter was sown to rye. The intermediate quarter served as a check plat. This practice was followed unchanged until recent years when the legume seeding was modified to include other legumes beside the crimson clover. Lime was added in 1899 at the rate of one ton of slaked lime, in 1911 at the rate of 1500 pounds of calcium oxide in slaked lime, and in 1914 at the rate of 1 ton of ground limestone (7).

FACTORS INFLUENCING THE REACTION

In the following paragraphs are considered the factors which operate on the various plats to influence the reaction of the soil.

A. The removal of soluble bases and acids by leaching. This depends mainly upon seasonal conditions. The leaching from the check plat may serve as an index of the magnitude of this factor, except as influenced by the cover crops.

B. The removal of acids and bases by cropping. The sizes of the crops removed during the first years of the experiment were not markedly different. The difference has become accentuated in later years.

The following statement shows the amounts of corn and stover removed from a quarter acre from 1897 to 1914:

	LEGUME PLAT	CHECK PLAT	RYE PLAT
Ear corn (bushels)	210 1	153 9	163 5
Stover (tons)	8 02	6 61	7 32

We may well ask the question as to the amounts of acids and bases removed by cropping, and what the variation has been in the various plats. The calculations are based upon the following estimated analysis of field-cured corn:

	EAR CORN	CORN STOVER
	per cent	*per cent*
Nitrogen	1 1	0 8
Phosphoric acid	0 6	0 4
Sulfuric oxide	0 3	0 2
Chlorine	0 1	0 5
Potassium oxide	0 4	1.3
Calcium oxide	0 2	0 5
Magnesium oxide	0 2	0 3

The acids are calculated from the nitrogen, phosphorus, sulfur and chlorine removed, and the bases from the potassium, calcium and magnesium. These are the most important elements in a consideration of the acids and bases. It is assumed that the nitrogen entered the plant from the soil fully oxidized, and that each atom of phosphorus was equivalent to an atom of calcium. Silicon is omitted because its reaction is so mild. The calculations show that an excess of acidic over basic ions was removed. The relation is brought out by the following:

Removal by corn, equivalent to pounds of calcium oxide

	ACIDS REMOVED	BASES REMOVED	EXCESS ACIDS OVER BASES REMOVED
1 bushel ear corn (70 pounds)	2 075	0 502	1 573
1 ton stover	49 04	33 82	15 22

Total excess acids over bases removed per quarter acre, 1897 to 1914

	LEGUME PLAT	CHECK PLAT	RYE PLAT
By ear corn	331	242	257
By stover	122	101	111
Total	453	343	368

These figures serve to indicate the amounts of soluble salts which were removed, and the relation of acids to bases. The difference between the check and other plats tends toward a conservation of soil bases and in reality, a reduction in the acidity. The reduction due to this consideration should be more marked on the legume area.

C. Addition of fertilizers. The addition has been the same on the three areas, except that beginning with 1915 the legume plat has received a smaller amount of nitrogen.

D. Decomposition of organic matter. Any difference in the plats may be attributed to this factor; that is, the fluctuations in the reaction on a given plat will be the resultant of the factors ($A + B + C + D$) which are being considered; A, B and C being factors operating about the same on the different plats while the factor D, due to the decomposition of organic matter, is the variable on which differences in the reaction of the legume and rye plats may be conceived to depend.

PLAN OF THE PRESENT EXPERIMENT

The plan as followed consisted in sampling the soils at various periods during the season to follow changes in acidity, and likewise in organic matter. It also seemed desirable to supplement the changes in the field with those which would occur in the laboratory.

Soil samples and treatment. Twelve borings 12 inches deep were taken from each plat with a soil sampler made of 2-inch pipe. The samples were brought to the laboratory and weighed, partially air-dried, mixed, sub-sampled and passed through a 2-mm. sieve. The determinations were made immediately on the partially air-dried soil. Moisture determinations were made and all analyses recorded on dry soil finer than 2 mm. Some of the soil was dried more thoroughly, and stored in sealed jars for work done subsequently.

Loss on ignition. The soil was dried at 100°C. for 24 hours and then ignited to constant weight. This loss on ignition includes, besides organic matter, especially water of hydration. It is believed, however, that on these soils the differences are largely attributable to the organic matter.

Reaction of the soil. (a) As indicated by lime-requirement methods. During the season of 1915 the MacIntire (13) and Jones (11) methods were used. In 1916 the ammonia method was used. Later the requirements of the stored samples were determined also by the ammonia method (10). (b) As measured by the concentration of hydrogen ions. In this work the colorimetric procedure of Gillespie was followed, using the sulfon ephthalein indicators (5) and phthalate buffer solutions of Lubs and Clark (3). These determinations were made only on the stored soil samples.

WORK ON THE FRESH SAMPLES TAKEN IN THE FIELD IN 1915

At the time of plowing for the corn in the spring of 1915 (May 18), the legume plat was well covered with vetch but the crimson clover was almost entirely winter-killed; the non-legume plat was well covered with rye. The average height of the rye at the date of plowing was 3 feet. The weight of the green crop from 1 square yard was 3.7 pounds of the legume and 1.3 pounds of the rye.

Soil samples were taken at frequent intervals from the date of plowing, and the lime requirements measured by the Jones and MacIntire methods. The fluctuations as indicated by these methods are recorded in table 1.

In table 1 it is seen that with the MacIntire method the requirement of the check plat increases slightly around the first of August and then diminishes so that the initial and final acidities are not very different. On the rye area the same mid-season increase takes place but the decrease in the latter part of the season is more marked and finally becomes quite decided. In opposition to the other two plats, the legume plat shows a decrease on August 13. This was followed by an increase so that the determinations compare fairly well in the fall with those recorded early in the season.

The Jones method detected only a slight variation, from which no positive statements are justified.

It has been shown in a former publication (9) that under closely regulated conditions the MacIntire method, as modified, would yield requirements in which the probable error of a given determination was 62.6 pounds CaO per

2,000,000 pounds of soil. With the Jones method, duplicate requirements varied from each other by 150 pounds CaO per 2,000,000 pounds of soil. By the ammonia method a number of determinations showed that the probable error could be reduced to 90 pounds of CaO per 2,000,000 pounds of soil.

The error in sampling the soil is shown by the fact that the range in the requirement of five samples taken from the legume plat on September 21, 1915, as measured by the MacIntire method, was 22 pounds CaO per 2,000,000 pounds of soil or well within the limits of error of the method itself.

TABLE 1

CaO *requirement per 3,000,000 pounds soil finer than 2 mm.*

DATE OF SAMPLING	MACINTIRE METHOD			JONES METHOD		
	Check	Rye	Legume	Check	Rye	Legume
1915	*lbs*	*lbs*	*lbs*	*lbs*	*lbs.*	*lbs.*
June 4	3276	3092	4175		1263	1620
June 10		3276	4242		1350	1738
June 21		3335	3966	1296	1350	1620
June 30	3393	3066	4301	1188	1361	1512
July 10	3150	3528	4536	1296	1296	1944
July 31	3486	2889	4578			
August 13	3612	3864	3270			
August 27	3237	2721	3723			
September 9	3360	1981	4253			
September 21			4091			

WORK ON THE FRESH SAMPLES TAKEN IN THE FIELD IN 1916

The crops on the legume plat were largely winter-killed, the alfalfa and sweet clover of the mixture were alive but had produced only a small growth. Fluctuations occurring on this plat, therefore, depended largely upon organic residues which had accumulated during the previous 20 years.

The non-legume plat was well covered with rye but the size of the crop was below normal. The average height was 2 feet. The green weight of rye, cut from 1 square yard just before plowing on May 18, was 1.4 pounds.

It was noticed in 1916 during the process of sampling that although the check and rye areas appeared quite similar and uniform, more variations existed on the legume plat. This plat slopes gently to the north and the variation in depth of surface soil is from 6.5 inches on the south to 9 inches on the north. The average depth of the surface soil of the three sections was:

	inches
Check	6 0
Rye	7.75
Legume	7 0

The weight of 1 cubic foot of soil, on May 9, 1916, was:

	pounds
Check	103 0
Rye	105 4
Legume	106.2

The weight of 15 borings, 12 inches deep (as sampled) was:

DATE OF SAMPLING	CHECK	RYE	LEGUME
1916	*lbs*	*lbs*	*lbs.*
April 25	31.0	29.5	28.0
May 2	30.5	29.5	27.8
May 9	31.0	29.0	29.0*
May 31	27.5	26.8	26.0

* 22.5 per cent moisture.

Table 3 shows during the field experiment an increase, or less decrease, in organic matter due to the cover crops, and that the tendency was toward a progressive decrease in organic matter from the date of plowing to the end of the season. If the averages are accepted as a criterion, it may be said that during the field experiment, the rye cover crops had increased the per cent of organic matter in the soil by 0.17, and the legume cover crops by 0.70.

TABLE 2

Variation in the per cent of soil finer than 2 mm.

DATE OF SAMPLING	CHECK	RYE	LEGUME
1916	*per cent*	*per cent*	*per cent*
April 13	92.4	92.9	92.5
April 25	96.0	96.5	95.9
May 2	92.5	96.0	94.1
May 9	93.1	96.9	96.7
May 31	93.3	95.9	95.2
June 5	93.2	95.1	96.7
June 14	92.8	94.1	97.1
June 20	94.2	95.6	97.1

TABLE 3

Differences in organic matter as indicated by the per cent of loss on ignition

DATE OF SAMPLING	CHECK	RYE	LEGUME
1916	*per cent*	*per cent*	*per cent*
April 13	4.31	4.52	5.25
May 18	4.46	4.56	5.04
May 31	4.42	4.62	5.20
June 5	4.37	4.64	5.00
June 14	4.40	4.57	5.02
June 20	4.43	4.53	5.10
July 7	4.33	4.49	4.95
August 24	4.25	4.42	4.97
Average	4.37	4.54	5.07

TABLE 4

Fluctuations in the acidity of the field samples soon after sampling, as determined by the ammonia method

DATE OF SAMPLING	REQUIREMENTS OF CaO PER 3,000,000 POUNDS OF SOIL		
	Check plat	Rye plat	Legume plat
1916	*lbs.*	*lbs.*	*lbs.*
April 13	3280	3140	5128
April 25	2954	2854	4266
May 2	3515	3603	5249
May 9	3649	3654	4924
May 18*	3295	3040	3814
May 31	3745	3866	5758
June 5†	3442	3247	4985
June 14	2954	2856	4435
June 20	3072	3073	
July 7	3226	3286	4423
August 24	3269	2994	4433

NOTE: The amounts recorded represent the average of triplicate determinations.

* Just before plowing.

† On this date the soil contained a large amount of ammoniacal nitrogen as indicated by the blank determination. It was then equivalent to an average lime requirement of 669 pounds. With the exception of May 31, other blanks were negligible.

DECOMPOSITION IN LABORATORY-TREATED SOILS IN 1916

The decomposition under field conditions is associated with a great many factors which are extremely difficult to estimate. Inasmuch as the size of the green crops returned to the soil was below normal in the spring of 1916, it seemed desirable to supplement the field studies with laboratory studies carried on at the same time, so that the application of green material could be increased and thereby accentuate any changes which might occur.

Accordingly, 102 borings 12 inches deep were taken from the check plat. The soil and subsoil were kept separate. Each of three Wiley pots, 12 inches in diameter and depth, was filled beneath with 26 pounds of subsoil and above with a layer of 35 pounds of surface soil.

One pot received 634 gm. of finely-cut, fresh red clover, of which 135 gm. were roots and 499 gm. were tops. Another pot received the same amount of rye in which the ratio of roots to tops was 3 to 5. The third pot received no organic matter. The mixtures were kept thoroughly stirred and a moisture content of 25 per cent was maintained.

The figures in table 5 indicate that during the period studied, rye produced an acid condition equivalent to about 500 pounds CaO per acre, over that indicated by the check pot. The pot receiving clover showed a slightly increased acidity but not so marked as that indicated by rye. Ammonification proceeded in the case of each green manure. The percentage of organic matter as indicated by loss on ignition was materially increased. The decomposition of clover proceeded more rapidly than that of rye.

TABLE 5

Fluctuations in the loss on ignition and in the acidity of the pot samples soon after sampling

DATE	TREATMENT	LOSS ON IGNITION	BLANK NH_3 IN 25 GM. SOIL	NH_3 RETAINED BY 25 GM SOIL — BLANK	CaO PER 3,000,000 POUNDS SOIL
1916		*per cent*	*mgm.*	*mgm.*	*lbs.*
May 25	Original soil	4.80	0.05	14.42	2848
July 25	Check	4.81	0.68	14.62	2888
	Rye	5.70	0.68	16.25	3210
	Clover	5.50	0.68	15.44	3050
September 12	Check	4.83	0.15	15.02	2967
	Rye	5.40	1.00	17.45	3447
	Clover	5.18	1.80	16.04	3168
December 16	Check	4.79	0.07	15.23	3008
	Rye	5.20	0.54	17.20	3397
	Clover	5.01	0.41	15.91	3143

SOIL CHANGES IN 1915 AND 1916 AS EXHIBITED BY THE DRIED STORED SAMPLES

This work on the samples collected over a period of two years shows that variations are maintained in the dried soils, as manifested in their ability to retain different amounts of ammonia. In the 1915 samples a difference existed between the initial and final acidity of the check as compared with those of the plats receiving organic matter, which relatively would credit the rye with increasing the requirement equivalent 637 pounds of CaO, while the increase due to the legume would be 942 pounds of CaO.

During the 1916 season the amount of green manure added in legumes was so slight that any variation in that year must be attributed to the addition of organic matter in former years. The rye plat exhibited a net increase of 65 pounds of CaO, while the legume plat recorded a net decrease of 236 pounds of CaO. Both quantities have little significance, inasmuch as they approach the error limits of the method, and because of fluctuations between near periods of sampling.

The net result over the period of 2 years indicates a decreased acidity except in the legume plat. The net difference between check and non-legume as compared with that between check and legume, suggests that the rye caused an increased requirement of 33 pounds while the legume caused an increase of 370. As has been stated, the last application of lime was made in the spring of 1914 in 1 ton of finely ground limestone per acre. This fact may have some significance when considering net changes in lime requirement even during 1915 and 1916.

TABLE 6

Fluctuations in acidity in soils stored over a period of 2 years, as indicated by the ammonia method

DATE OF SAMPLING	REQUIREMENT OF CaO PER 3,000,000 POUNDS OF SOIL FINER THAN 2 MM.		
	Check plat	Rye plat	Legume plat
1915	*lbs*	*lbs.*	*lbs.*
June 4	2686	2518	3559
June 10		2988	3962
June 21	2686	2821	3559
June 30	2250	2921	3727
July 10	2418	3056	3761
July 31	2250	2753	3559
August 13	2686	4063	4101
August 27	2418	2586	3559
September 21	1914	2383	3729
1916			
April 13	2383	2183	3862
April 25	2047	2418	3358
May 2	2317	2686	3761
May 9	2250	2686	3425
May 19	2383		3358
May 31	2921		
June 5		2686	
June 14	2921	2686	4154
June 20	2388	2753	3559
July 7		2988	3727
August 24	2518	2383	3761

TABLE 7

Seasonal variation in the CaO *requirement and the relation of final to initial requirement, as given in table 6*

SEASON	FLUCTUATION IN CaO REQUIREMENT			FINAL, AS COMPARED WITH INITIAL CaO REQUIREMENT		
	Check	Rye	Legume	Check	Rye	Legume
	lbs	*lbs.*	*lbs*	*lbs*	*lbs*	*lbs*
June 4 to September 21, 1915	772	1680	600	−772	−135	170
September 21, 1915, to April 13, 1916				469	−200	133
April 13 to August 24, 1916	874	805	796	135	200	−101
June 4, 1915 to August 24, 1916				−168	−135	202

THE HYDROGEN-ION CONCENTRATION IN THE SOILS

It is to be regretted that the true reaction of the soil solution, as measured by the hydrogen-ion concentration, has not received more attention. The question may well be raised, however, as to the relation which the hydrogen-ion concentration, measured by making an aqueous extract in colorimetric work, and a suspension in electrometric work, bear to the concentration of hydrogen ions in the true soil solution.

Whatever the relation may be, it is obvious that if a high concentration of hydrogen ions is recorded in the aqueous extract, the concentration will be as great or greater in the true soil solution with which the plant is associated.

TABLE 8

Hydrogen-ion concentration in the soils after storage

DATE SAMPLED	SOIL	pH	H'
	Field soils		
1915			
June 4	Check	6.0	1.0×10^{-6}
July 10	Check	6.0	1.0×10^{-6}
August 13	Check	6.0	1.0×10^{-6}
September 9	Check	6.0	1.0×10^{-6}
June 4	Rye	6.1	0.8×10^{-6}
August 13	Rye	5.8	1.6×10^{-6}
September 9	Rye	6.4	0.4×10^{-6}
June 4	Legume	6.0	1.0×10^{-6}
July 31	Legume	5.9	1.2×10^{-6}
August 13	Legume	6.0	1.0×10^{-6}
September 21	Legume	6.0	1.0×10^{-6}
1916			
April 25	Check	6.2	0.6×10^{-6}
May 31	Check	6.1	0.8×10^{-6}
June 14	Check	5.9	1.2×10^{-6}
August 24	Check	6.1	0.8×10^{-6}
April 13	Rye	6.3	0.5×10^{-6}
June 14	Rye	5.9	1.2×10^{-6}
July 7	Rye	6.0	1.0×10^{-6}
August 24	Rye	6.2	0.6×10^{-6}
May 19	Legume	5.8	1.6×10^{-6}
June 5	Legume	5.8	1.6×10^{-6}
June 14	Legume	5.9	1.2×10^{-6}
August 24	Legume	6.0	1.0×10^{-6}
	Pot-treated soils		
1916			
December	Check	5.9	$1.2 - 10^{-6}$
	Rye	5.9	$1.2 - 10^{-6}$
	Legume	5.9	$1.2 - 10^{-6}$

The results here recorded in table 8 are with the dried soils, and consequently an acidity due to the easily volatile acids, which might have been measured in the moist soil at the time of sampling, does not enter into the reaction.

It is seen that the concentration of hydrogen ions from the three plats sampled in 1915 shows variations in pH of from 5.8 to 6.4. The greatest variation occurs in samples from the rye plat, the other two plats remaining more constant. It is significant that with the rye plat the variations in concentration of hydrogen ions, follow in a general way the fluctuations in the lime requirement.

In 1916 a midsummer increase as compared with final acidity is seen in each soil, and the legume plat was the only one to show a higher initial than final acidity.

The final reaction of pot-treated soils appears to have been unaltered by the addition of organic matter.

GENERAL DISCUSSION

In the absence of records of soil temperatures and soil moisture, the mean air temperature and the precipitation may be considered briefly in connection with the acidity fluctuations in the field soil, relying chiefly on the determinations obtained with the fresh soil. In 1915, 8 days prior to taking the first sample on June 4, there had been no rain and an average mean temperature of 48°F. June had an average mean temperature of 59° and a precipitation of 1.36 inches, accompanied by no marked changes in acidity. During the first 9 days in July more rain fell than during all of June but the mean temperature had not changed much. A small increase in acidity was general. There was only 0.72 inch of rainfall during the remainder of July, and on the thirty-first, as a rule, no marked change in acidity was recorded. In the first 12 days of August, 5.2 inches of rain fell, accompanied by a mean temperature of 63°. On August 13, with the exception of the legume plat, which had already reached the maximum, the highest acidity was recorded, and by the twenty-seventh the tendency was generally downward so that in no case was there a greater acidity at the close of the season, which was dry, than at the beginning.

In 1916, the sampling was begun much earlier than in 1915, namely, on April 13, as soon as the frost was out of the soil. There was a fair prospect with the rye, but the legumes were practically a failure. The lime requirement of the legume plat proved to be about a ton of calcium oxide greater than that of the other two plats. During the succeeding 12 days, 2.6 inches of rain fell and the acidity dropped a few hundred pounds, which may have been due to leaching. Although during the following 7 days 1.7 inches of rain fell, the previous drop in acidity had been fully offset, as is shown by the determinations of May 5 and 9. There were 4.6 inches of rainfall during the remainder of the month, over an inch of which fell 2 days before the May 31 samples were taken. The highest acidity of the summer had then been reached; whereas, the lowest acidity and highest ammonia formation was recorded on June 5, about another inch of rain having fallen in the meantime. The temperature between May 24 and June 4 was fairly uniform, with a mean of 57°. The plats were plowed

May 18, fertilized May 23, and planted May 25. Samples of soil were also collected on June 14 and 20 and July 7, but only a moderate increase in acidity was revealed by their examination. Over 6 inches of rain had fallen during the period since June 4, and heavy rainfall continued throughout the remainder of July. The driest period of the summer was during the last half of August. The mean temperature in August was 65°. One more lot of samples was taken on August 20 but not much change in acidity was registered. There had been no net increase in acidity over that shown on April 13. Below are given the average of the mean daily temperatures, and the rainfall in inches during the warmest four months.

		MAY	JUNE	JULY	AUGUST
Mean temperature (°)	1915	50.5	59.4	65.1	63.7
	1916	51.0	56.7	64.0	65.0
Precipitation (in.)	1915	2.64	1.36	2.28	7.74
	1916	4.80	5.77	11.75	2.77

In 1915 the highest acidity was in the August 13 samples; and in 1916 it was in the May 31 samples.

No conclusions seem to be warranted from the limited data at hand regarding the seasonal fluctuations. Possibly a liberal rainfall but not enough to cause a leaching of acid substance from the surface foot of soil is conducive to high acidity (4).

If the determinations on the stored samples of the two years, which were analyzed by a uniform method, are used as a criterion, it may be seen that there was practically no net increase in acidity during the years 1915 and 1916, finely ground limestone having been applied in 1914. In fact, there is no evidence that any acidity has resulted from the use of rye as a cover crop for a quarter of a century. The legumes, however, have during the same time, considerably increased the lime requirements.

In the uncropped soils treated in the laboratory with an equal weight of green rye as compared with clover, the rye increased the lime requirement 300 to 400 pounds, or about twice as much as the clover, and the increase was maintained from July to December.

It is evident that the results of the work on samples collected during 1915 and 1916, taken together, were scarcely an index of the net cumulative effect exerted by the cover crops through the longer period of time.

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SOME FACTORS INFLUENCING THE QUANTITATIVE DETERMINATION OF CHLORIDES IN SOIL

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The advancements made in any field of endeavor are limited by the rapidity with which methods are developed in that subject. The value of the results in any experiment is directly proportional to the accuracy of the method used. It is useless to plan and conduct long and costly experiments when the accuracy of the analytic method is questionable or unknown. Hence, one need offer no apology for work, the object of which is the perfection of new or the study of old analytical methods. Moreover, methods have often been devised and tested for use on one substance or under one set of conditions and may or may not give good results when used on other substances and under other conditions.

Methods have been devised for the determination of chlorine in water, milk, blood, urine, soil and other naturally-occurring substances. No two of the methods give equally reliable results under all conditions. Hence, this investigation was designed to study and select the one best suited for use in the determination of chlorides in soil.

An ideal method for the determination of chlorides in soil must be accurate when applied to substances containing little or much chlorine. It must be accurate when used on soils having large quantities of other soluble salts and those containing large amounts of organic matter. It should be fairly easy of manipulation and of such a nature that a great number of determinations can be made in a short time.

The gravimetric method is not suitable for the determination of chlorides in soil on account of the time it consumes where so many determinations are to be made. Moreover, waters and solutions which contain very small amounts of chlorides do not form coagulated precipitates unless first concentrated by evaporation. Certain substances have a solvent action on silver chloride when heated, among them being the nitrates of the alkali and alkali earth metals (5, p. 199) and silver nitrate in strong solution. Moreover, heating the chloride solution with nitric acid before the addition of the precipitating agent, as is usually done, results in a loss of chlorides (5, p. 522).

In the determination of alkali or alkali earth chlorides volumetrically by Mohr's method, the solution must be absolutely free from acid or any great excess of alkali. It is best to have them neutral and cold (13). Yoder (8) states that there should be no carbonates or phosphates present, as the silver salts of these acids are less soluble than the chromate, and the solution should have a certain definite concentration in order to give the most definite color change. If the chlorine content is below 7.5 parts per million the solution should be concentrated (14). Kolthoff (7) found that 0.7 to 1 cc. of 1 *N* potassium chromate per 100-cc. sample was the best amount of indicator to use and that the H-ion concentration could vary between 5×10^{-7} and 5×10^{-11}. He found also that the harmful effects of phosphates can be avoided by using more indicator, that borax, SO_4 and NH_3 below 50 mgm. per liter are harmless, and that proteins and iron salts must be absent. Winkler (17) states that a comparison should be made by running duplicate samples. To one silver nitrate is added until a faint red color is produced which is then just discharged with sodium chloride. To the other silver nitrate is added until a faint red color is formed and persists for five minutes. A correction is subtracted from the burette reading, the amount of the correction increasing with the amount of silver nitrate used. Kolthoff (7) denies that the correction increases with the amount of chlorine, as claimed by Winkler.

Tillman and Heublein (14) claim the Mohr method gave too high results when too little indicator is used, the titration being carried too far. If sufficient indicator is used no correction is necessary. They state that at least 1 cc. of a 10 per cent solution of potassium chromate should be used for each 100 cc. of solution.

An error may be introduced by a rise in temperature since the solubility of silver chromate increases with a rise in temperature (16). The presence of nitrates also increases the solubility of silver chromate.

For small amounts of chlorine in concentrated solutions the Mohr method gives very definite results. If, however, the volume of the solution is too large the results are not very accurate. In all cases the sensitiveness of the indicator should be tested by determining the quantity of silver solution necessary to produce the red shale used in the titration when no chlorides are present.

When a decinormal solution of silver nitrate is used an excess of about 0.2 cc. is required and this must be subtracted from the amount used. The color change is sharper when sodium arsenate is used as indicator and no correction is then necessary (8). Stuart (12) found the Mohr method could not be used on peaty waters.

In the original Volhard method the excess of silver nitrate was titrated with the alkali thiocyanate in the presence of precipitated silver chloride without any special treatment. This gives results which are satisfactory with large quantities of chlorides, but which are too high with small quantities (15). Drechsel (4) shows this to be due to the silver thiocyanate, being less soluble than the silver chloride, so that on stirring the red ferric thiocyanate gradually decomposes, forming silver thiocyanate and making it difficult to obtain the true end-point, since the red color becomes permanent only after considerable excess of thiocyanate has been added. He overcame this difficulty by adding an excess of silver nitrate to the chloride solution contained in a graduated flask and shaking until the silver chloride coagulated, then filtering through a dry filter, adding the indicator to an aliquot part and titrating the excess of silver nitrate with the thiocyanate solution.

Volhard in his original work contended that the velocity of decomposition of ferric thiocyanate was so slow that it would not affect precise work. But Rosanoff and Hill (9) showed it to be rather high, causing an error in some cases of as much as 2.9 per cent where the silver chloride is left in the solution, whereas when it is removed before the addition of the sulfocyanate the error does not exceed 0.17 per cent.

The Volhard method, without the removal of the silver chloride, is accurate only when there is a rather large amount of chlorides present and when the solution is not too dilute. When the silver chloride has been thoroughly coagulated by boiling after the addition of an excess of silver nitrate, it reacts less readily with the thiocyanate and it is possible to obtain correct results after the solution has been cooled without its removal (10). Silver chloride may be as well coagulated by the addition if ether as by boiling, and under these conditions the chlorides may be correctly determined by the Volhard method without the removal of the precipitated silver chloride (1). In our work we found that alcohol was just as efficient as the ether or boiling.

TABLE 1

Chlorides obtained by the Volhard method when the solution is heated and treated with alcohol or ether

TREATMENT	NUMBER OF DETERMINATIONS	CHLORIDES CALCULATED AS NaCl	PROBABLE ERROR
		per cent	
Heated to boiling	3	4 944	±0 01
10 cc ether	4	4 937	±0 007
10 cc ethyl alcohol	4	4 937	±0 007

The results obtained by us after coagulating the chlorides in soil solution by the various methods are given in table 1.

The differences in the various results are well within the experimental error, hence the ether and the alcohol are just as efficient coagulants as is heat. Moreover, the use of ether or alcohol saves the time used in heating and cooling the solution. For these reasons we have used alcohol as the coagulating agent in our work.

From the preceding brief review of the literature it would appear that the Mohr and Volhard methods are best suited for the determination of chlorides in soil, hence our experimental work has been based on them.

Preparation of a clear filtrate. The first prerequisite in the determination or chlorides in the soil is the preparation of a clear solution. Investigations have already been carried out in this laboratory showing the relative efficiency of the Pasteur-Chamberland filters, centrifuge, calcium oxide, calcium carbonate, talc, ferric sulfate, potassium alum, sodium alum and iron alum as clarifying agents, and also their effects on the determination of nitric nitrogen in the soil solution (6).

Of these the potassium alum, the Pasteur-Chamberland filter and the centrifuge gave the most satisfactory solutions and interfered the least with the determination of the nitric nitrogen in the solution.

In the present work, therefore, these three methods were used for obtaining the solution in which the chlorides were to be determined. The results obtained by the various methods are given in table 2.

The soil used was a heavy black clay loam collected from a depression about four miles west of Corrinne, Utah. Evaporation had left the soil with a thick incrustation of alkali salts. The soil was dried in the laboratory, pulverized, sieved, and preserved in wooden boxes.

TABLE 2

Chlorides obtained from soil solutions which were clarified by the filter, the centrifuge or alum

TREATMENT	CHLORIDES	ERROR	NUMBER OF DETERMINATIONS
	per cent		
Filter. .	4 998	±0 011	18
Centrifuge	5 02	±0 013	18
Alum	4 998	±0 01	18

In so far as these results show, there is nothing to choose between the filter and the alum since they give concordant results and the probable error is about the same in each case. The centrifuge, however, gives higher results. It is possible that colloids or proteins from the soil were present and remained in suspension with the centrifuge and would be removed with the alum and filter. It is a fact that the centrifuge at the speed at which we run it, about 1200 revolutions per minute, always gave a murky, cloudy solution, whereas the filter and the alum gave perfectly clear solutions.

It seems, therefore, that the filter or the alum where permissible is the best means for obtaining a clear filtrate. This is in keeping with our finding on the preparation of a clear solution for the determination of nitric nitrogen in the soil solution, where we found that alum and the filter gave clear filtrates and interfered least with the recovery of the nitric nitrogen.

The amount of chlorides in the soil. In order that we may have some standard of comparison it was necessary to know as accurately as possible the amount of chlorides in the soil. For this reason six 10-gm. portions were leached free of chlorides. The filtrate was made to 500 cc. and aliquot parts analyzed. The gravimetric method which is recognized as giving very accurate results under proper conditions gave, as an average of 11 determinations, 4.98 per cent of chlorides calculated as sodium chloride. These determinations agreed within experimental error, hence may be taken as the per cent of chlorides in the soil.

The adsorptive action of filters on sodium chloride. The adsorptive action of Pasteur-Chamberland filters on sodium chloride was determined by filtering solutions of sodium chloride of varying strengths. These solutions were then titrated with standard 0.1 *N* silver nitrate.

The quantity of chlorides in the solution is the same after filtration as before, thus indicating that any adsorptive action which may be exerted by the filter is within experimental error, a conclusion which is in accord with other findings (3, 6).

TABLE 3

Sodium chloride in solutions before and after filtering through Pasteur-Chamberland filters

MOHR METHOD		VOLHARD METHOD	
Before filtration	After filtration	Before filtration	After filtration
mgm.	*mgm.*	*mgm.*	*mgm.*
5 855	5 878	5.867	5 859
2 937	2 937	2 931	2 955
1 962	1 971	1 952	1 962
1 488	1 488	1.471	1 483
1 183	1 181	1 184	1 161

TABLE 4

The chlorides, calculated as sodium chloride, extracted from soils with varying amounts of water

[illegible]	CHLORIDES EXTRACTED FROM SOIL BY VARYING RATIOS OF WATER AND DETERMINED BY			
	Mohr method	Error	Volhard method	Error
	per cent		*per cent*	
1 to 5	5 041	±0 008	4 975	±0 012
1 to 10	5 067	±0 01	4 986	±0 012
1 to 15	5 054	±0 013	4 956	±0 012
1 to 20	5 057	±0 017	4 974	±0 013

Ratio of soil to water. For the extraction of soluble salts from soils the workers have used ratios as narrow as 1 part of soil to 1 of water, and as wide as 1 part of soil to 5 parts of water. In our work the following ratios were used: 1 to 5, 1 to 10, 1 to 15 and 1 to 20, with the results reported in table 4.

The soil yielded to the gravimetric method 4.98 per cent of chlorides, calculated as sodium chloride. Here we find when the ratio of soil to water is 1 to 5 by the Volhard method 4.975 per cent and with the ratio 1 to 20, 4.974 per cent. The Mohr method gave 5.041 and 5.067, respectively, for the same ratios. The per cent of chlorides found in the soil by leaching is

4.984. The average of that found by the Mohr and Volhard methods for the ratios 1 to 5 and 1 to 20 are 5.008 and 5.037 per cent, respectively, which are slightly higher than those yielded on leaching the soil, the difference probably being experimental error. The Volhard method, however, yielded results which are almost identical with those obtained on leaching and determining gravimetrically the chlorides. We may conclude that all the chlorides are extracted by using 5 parts of water to 1 of soil. In our work on the extraction of nitric nitrogen from soils (6), we found this ratio of water to soil sufficient to extract all nitrates present.

Time of extraction. The time necessary for water added to a soil to dissolve the chlorides in a soil may vary greatly with the conditions of the soil when the water is added, as well as the treatment of the soil-water mixture.

In order to find the minimum time necessary for extraction we have extracted 100-gm. portions of soil containing 2 gm. of alum with 500 cc. of water. The soil was mixed with the water in 1000-cc. bottles and shaken in a mechanical shaker for the time indicated, then allowed to stand long enough to settle and then the chlorides determined. The average results are given in table 5.

TABLE 5

Chlorides extracted from a soil after varying periods

TIME OF SHAKING	GENERAL AVERAGE		THE SOLUTION CLARIFIED WITH 2 GM ALUM		SOLUTION CLARIFIED BY CHAMBERLAND-PASTEUR FILTERS	
	Sodium chloride	Error	Sodium chloride	Error	Sodium chloride	Error
minutes	*per cent*		*per cent*		*per cent*	
5	4 987	±0 015	5 002	±0 01	4 973	±0 021
10	4 997	±0 007	5 002	±0 005	4 993	±0 009
20	4.981	±0 011	4 969	±0 013	4 994	±0 01
30	4 992	±0 009	4 990	±0 01	4 994	±0 008
45	4 977	±0 012	4 970	±0 011	4 984	±0 012

If we take as the amount of chlorides present, that shown by the gravimetric method on the solutions obtained by leaching the soil free of chlorides, namely, 4.98 per cent, then there is nothing to be gained by shaking the soil longer than five minutes, as may be observed from the results which all agree within experimental error. This is probably sufficient where the soil is finely divided and thoroughly agitated as was the case with this soil.

The influence of various salts on the determination of chlorides in soil by the Volhard and the Mohr methods In order to ascertain the influence of salts on the determination of chlorides in soil solution, 50 gm. of soil to which was added 5 gm. of the various salts was agitated with 500 cc. of water in a shaking machine for 10 minutes. Part of the solutions were filtered through Pasteur-Chamberland filters and part clarified by the addition of 2 gm. of alum. The chlorides were determined in aliquot portions by both the Mohr and Volhard methods. The results are given in table 6. It will be noticed that the salts are tabulated in such a way as to show the influence of the anion and the cation. The calcium salts give normal results both by the Mohr and the Volhard methods, that is, the results are entirely comparable with those obtained by the same method in the absence of the salts. The same is also true of the magnesium ion. With the exception of the sodium

TABLE 6

The influence of various salts on the determination of chlorides by the Mohr and Volhard methods

METHOD	SALT USED	ALUM			FILTER		
		Number of analyses	Error	NaCl	NaCl	Error	Number of analyses
				per cent	*per cent*		
Volhard	$CaCO_3$	6	±0.007	5.01	5.02	±0.01	6
	$Ca(NO_3)_2$	6	±0.004	4.99	4.95	±0.02	6
	$CaSO_4$	6	±0.003	5.02	5.01	0.0	6
Average		18	±0.005	5.007	4.993	±0.01	18
Mohr	$CaCO_3$	6	±0.003	5.08	5.12	±0.01	6
	$Ca(NO_3)_2$	6	±0.008	5.08	5.03	±0.02	6
	$CaSO_4$	6	±0.005	5.09	5.09	±0.00	6
Average		18	±0.005	5.08	5.08	±0.01	18
Volhard	$MgSO_4$	4	±0.01	4.99	5.02	±0.002	6
	$Mg(NO_3)_2$	9	±0.008	4.942	4.925	±0.003	6
	$MgCO_3$	5	±0.01	4.946	4.954	±0.01	6
Average		18	±0.009	4.957	4.954	±0.005	18
Mohr	$MgSO_4$	4	±0.00	5.09	5.08	±0.002	6
	$Mg(NO_3)_2$	6	±0.006	5.018	5.004	±0.003	6
	$MgCO_3$	6	±0.01	5.028	5.054	±0.01	6
Average		16	±0.005	5.045	5.046	±0.005	18
Volhard	Na_2CO_3	6	±0.018	4.89	4.95	±0.00	6
	$NaNO_3$	6	±0.01	4.87	4.88	±0.02	6
	$NaHCO_3$	6	±0.008	4.90	4.91	±0.005	6
	Na_2SO_4	6	±0.012	4.92	4.93	±0.01	6
Average		24	±0.012	4.895	4.92	±0.008	24
Mohr	Na_2CO_3	6	±0.00	5.26	5.26	±0.00	6
	$NaNO_3$	6	±0.007	4.99	4.95	±0.02	6
	$NaHCO_3$	6	±0.01	5.02	5.02	±0.025	6
	Na_2SO_4	6	±0.016	5.03	5.02	±0.005	6
Average		24	±0.008	5.075	5.062	±0.014	24

TABLE 6—*Continued*

METHOD	SALT USED	CLARIFIED WITH ALUM			CLARIFIED WITH FILTER*		
		Number of analyses	Error	NaCl	NaCl	Error	Number of analyses
				per cent	*per cent*		
Volhard	$CaCO_3$	6	±0 007	5 01	5 02	±0 01	6
	$MgCO_3$	5	±0 01	4 946	4 954	±0 01	6
	Na_2CO_3	6	±0 018	4 89	4 95	±0 00	6
	$NaHCO_3$	6	±0 008	4 90	4 91	±0 005	6
	$FeCO_3$	6	±0 005	4 938	4 967	±0 002	6
Average		29	±0 009	4 938	4 960	±0 005	30
Mohr	$CaCO_3$	6	±0 003	5 08	5 12	±0 01	6
	$MgCO_3$	6	±0 01	5 025	5 054	±0 01	6
	Na_2CO_3	6	±0 00	5 26	5 26	±0 00	6
	$NaHCO_3$	6	±0 01	5 02	5 02	±0 005	6
	$FeCO_3$	6	±0 00	5 042	5 045	±0 002	6
Average		30	±0 005	5 085	5 102	±0 005	30
Volhard	$Ca(NO_3)_2$	6	±0 004	4 99	4 95	±0 02	6
	$Mg(NO_3)_2$	9	±0 008	4 942	4 925	±0 003	6
	$NaNO_3$	6	±0 01	4 87	4 88	±0 02	6
Average		21	±0 007	4 934	4 918	±0 014	18
Mohr	$Ca(NO_3)_2$	6	±0 008	5 08	5 03	±0 02	6
	$Mg(NO_3)_2$	6	±0 006	5 018	5 004	±0 003	6
	$NaNO_3$	6	±0 007	4 99	4 95	±0 02	6
Average		18	±0 007	5 029	4 995	±0 014	18
Volhard	$CaSO_4$	6	±0 003	5 02	5 01	±0 00	6
	$MgSO_4$	4	±0 01	4 99	5 02	±0 002	6
	Na_2SO_4	6	±0 012	4 92	4 93	±0 01	6
Average		16	±0 008	4 977	4 99	±0 004	18
Mohr	$CaSO_4$	6	±0 005	5 09	5 09	±0 00	6
	$MgSO_4$	4	±0 00	5 09	5 08	±0 002	6
	Na_2SO_4	6	±0 016	5 03	5 02	±0 01	6
Average		16	±0 007	5 07	5 063	±0 004	18

carbonate, which gives abnormally high results, the sodium salt decreases the quantity of chlorides recovered.

Of the acid ions the sulfates are without effect whereas the nitrates have a depressing influence. This is in conformity with the reference already cited (5, p. 522) that silver chloride is somewhat soluble in the presence of nitrates of the alkali and alkali earths. The carbonates decreased the quantity of chloride as measured by the Volhard method. This is to be expected, since the carbonates are converted into nitrates by the added nitric acid. With the exception of sodium carbonate the carbonates do not interfere with the Mohr method. This is undoubtedly due to the low solubility of the carbonates used. With sodium carbonate, however, abnormally high results are obtained. Besides coloring the filtrate brown, thereby obscuring the end-point, sodium carbonate causes a precipitation of silver carbonate, since silver carbonate is less soluble than silver chromate and the amount of silver carbonate precipitated will depend, of course, on the quantity of sodium carbonate present.

The Mohr vs. the Volhard Method. Brautlecht and Langley (2) compared these methods in their adaptability to the determination of chlorides in waters. They found the Volhard gave more satisfactory concordant and accurate results when 0.1 *N* solutions were used but required a longer time. They found 0.01 *N* sulfocyanate and silver nitrate solutions too dilute to give sharp end-points. Shutt and Charlton (11) reached a similar conclusion when working on a large number of waters containing only small amounts of chlorine. Stuart (12) found that the Volhard method can be used with waters containing small amounts of chlorine only when a very small excess of silver nitrate is used, not exceeding 0.2 cc. The plus or minus error will be about 0.5 parts per million of waters containing 10 parts per million of chlorine.

TABLE 7

Chlorides found in solution by the Mohr and Volhard methods

MOHR METHOD		VOLHARD METHOD	
Chlorides calculated as NaCl	Error	Chlorides calculated as NaCl	Error
per cent		*per cent*	
5 041	±0 011	4 964	±0.011

The vast amount of work done by us makes it possible to compare the results obtained by the Mohr and Volhard methods with those obtained by the gravimetric.

This is done in table 7, in which is given the average for 22 determinations by the two methods. The determinations were made on the same solutions so that the only variable is the method of determining the chlorides.

The Mohr method invariably gives results higher than the Volhard method. This has been rectified by many workers by making a correction which is not necessary in the case of the Volhard method.

If we take the chlorides as determined by leaching and precipitation with silver nitrate (4.98 per cent) as standard we find a very close agreement between this result and that obtained by the Volhard method. Therefore, it would appear from all of our results that the Volhard method is more accurate for the determination of chlorides in soil solution than is the Mohr method.

SUMMARY

Equally satisfactory soil extracts from which the chlorides are to be determined are obtained either by the use of 2 gm. of alum or by the Pasteur-Chamberland filter. The extract obtained by use of the centrifuge gave higher results, due perhaps to the presence of proteins or other colloids in suspension. The quantity of chlorides obtained by the use of 5 parts of water to 1 part of soil was the same as with larger quantities of water.

Nothing is to be gained by agitating the soil and water for more than 5 minutes if the soil is finely divided and the solution vigorously shaken. The Mohr and Volhard methods give low results in the presence of nitrates of the alkali and the alkali earths. The Volhard method gives low results in the presence of carbonates of the alkali and alkali earths. The Mohr method gives high results in the presence of carbonates due to the precipitation of silver carbonate. The Volhard method gives more concordant and, as judged by the gravimetric method, more nearly exact results than the Mohr method. Ethyl alcohol causes the coagulation of silver chloride as well as ether or boiling.

We obtained the best results by the following method: 100 gm. of finely pulverized soil was agitated in a mechanical shaker for 5 minutes and clarified either by the use of 2 gm. of alum or by the Pasteur-Chamberland filter; 20-cc. portions were pipetted into ground-glass-stoppered bottles and an excess of 0.1 *N* silver nitrate added; 10-cc. of 95 per cent ethyl alcohol was added and the mixture shaken until the silver chloride was coagulated and the supernatant solution was clear; 2 cc. of ferric sulfate solution containing 20 gm. of ferric sulfate to 500 cc. of water, and 5 cc .of dilute nitric acid were added; the excess of silver nitrate was then titrated by the use of 0.1 *N* NH_4SCN.

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THE FORMATION OF NITRATES IN A SOIL FOLLOWING THE GROWTH OF RED CLOVER AND OF TIMOTHY

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It has usually been observed that the growth of clover or alfalfa on a piece of land improves it in so far as crop production is concerned. This is true even when the hay crops are all removed and only the stubble and roots incorporated in the soil. Because these plants are nitrogen fixers, we are naturally inclined to associate the improved productiveness to an increase in the supply of total or available nitrogen.

There still remain several questions regarding the reason for soil improvement by legumes which have not been definitely settled. There appears to be some doubt as to whether the total nitrogen content of a soil is always greater after a legume has been raised on it than before, even when its growth has made the soil capable of producing larger crops. If there has been no increase in the quantity of nitrogen present, has there been a change in the readiness with which organic nitrogen is converted into nitrates, and if so, is that due to the quality of the organic nitrogen in the plant residues or does it result from some other influence of the legume on the production of nitrates?

It is not our purpose to discuss in this paper the effect of legumes on the nitrogen balance in soil on which they have been grown. It is, rather, the effect of these crops on the quantity of nitrogen in the form of nitrates after the legumes have been cut for hay and the stubble or roots plowed under that is here to be considered. Experiments reported by a number of investigators cover two aspects of the subject. One of these is the capacity of a soil to form nitrates from the nitrogen remaining in the soil after the growth of the legumes; the other is the power of the soil to form nitrates from organic nitrogenous matter or ammonium salts added to the soil when it is brought into the laboratory and incubated.

In the following brief review of some of the investigations dealing with this subject no consideration is given to records of the determination of nitrates in soil on which crops are growing unless samples of soil are removed and nitrates allowed to develop during incubation of the soil without the presence of crop plants. The presence of growing crops introduces a factor which complicates any attempt to find the effect of the residues of the crop on nitrate formation. To simplify the discussion this limitation will be observed, and the effect of fallow or condition other than crop growth will be omitted.

Warington (10) analyzed the drainage water passing through a soil from which a wheat crop had been removed, one-half of the area having grown a crop of clover immediately before the wheat was planted and the other half having been in barley. He found that the drainage from the clover soil contained more nitrate nitrogen than did the barley soil.

King and Whitson (6) conducted experiments with soil in cylinders in the greenhouse which they planted respectively to corn, clover and oats. After these crops were removed the cylinders were allowed to stand for 93 days under the same conditions of moisture and temperature. During this period one set of cylinders representing the three crops was left uncultivated, one set was cultivated once a week, and one set was cultivated once in two weeks. At the end of the period the quantity of nitrates in the cultivated soil was in the ratio of 31: 47: 28 for corn, clover and oats, respectively, but in the uncultivated soil there was practically no difference in the quantity of nitrates in the soil previously planted to these crops.

As the method of treating the sample of soil previous to the determination of nitrates may possibly be a factor in the results, that operation will be described in each review if it has been found in the text. In this experiment the determination of nitrates appears to have been made without previously drying the soil.

Brown (1), in experimenting with soil from a series of field plats which had been cropped in various ways for a number of years, found that incubation with ammonium sulfate and also with dried blood gave, on the whole, a slightly lower production of nitrates from the soil on which clover had been grown continuously than from soil on which corn had been grown continuously, but where clover had been used in a rotation nitrate production was much higher than in the soils just mentioned and also than in soil on which the same rotation was used without clover.

In these experiments and also in those by Brown to be reviewed later the soil before incubation was air-dried and screened.

Greaves (3) determined the nitrifying power of soil from a number of fields whose previous treatments had been very diverse but which at the time of sampling were planted to alfalfa and wheat. He reports that the nitrifying power of soil on which alfalfa was growing was much less than that of soil on which wheat was growing.

In a continuation of the experiment by Brown (2) previously reviewed he found that both dried blood and ammonium sulfate produced more nitrate nitrogen when incubated with soil cropped to a rotation of corn, oats and clover than with a soil cropped only to corn and oats, or soil planted continuously to corn.

McBeth and Smith (8) experimented with soils from plats irrigated with different quantities of water and also unirrigated. Each set of plants so treated produced crops of alfalfa, potatoes, corn and oats. The experiment extended through the years 1909 to 1912, inclusive. The nitrifying power of the soil with dried blood and also with ammonium sulfate was determined. Soil samples were air-dried to 3 per cent moisture before the first determination of nitrates was made and before the sample was incubated. While the results of the nitrification tests were not uniform for the different years and the different applications of irrigation water they showed, in the main, a greater nitrifying power for the alfalfa soil than for the potato, corn and oat soil, which three crops appear not to have differed much in their effect on the nitrifying power of the soil.

Velbel (9) found that nitrate formation was increased by the cultivation of leguminous plants.

Kellerman and Wright (5) planted peas, vetch, barley, rye and Kafir corn in wire baskets, using a series of these crops in each of two types of soil. After growing for 80 days the plants and roots were removed and nitrification tests were made with the addition of peptone to one set of soil samples and of ammonium sulfate to another set. There appeared to be no consistent difference between the soil planted to the legumes and that planted to the non-legumes in respect to the quantity of nitrates formed during incubation.

Greaves, Stewart and Hirst (4), experimenting apparently with the same plats at Greenville, Utah, as those used in the experiments by McBeth and Smith, which have already been reviewed, and following much the same methods of preparing soil for incubation, found that the nitrifying power of soil with dried blood was greatest when taken from where potatoes were growing and less for alfalfa, oats and corn in the order named.

Lyon and Bizzell (7) found more nitrate nitrogen in soil on which alfalfa had previously grown for 6 years and which was then kept bare of vegetation than in soil on which timothy had grown for the same period and which was also free of vegetation. They also found that the alfalfa soil produced more nitrates when incubated both with and without dried blood.

There is sufficient discrepancy in the results of the experiments reported to leave some doubt whether the legumes used in the experiments increased the nitrifying power of the soil for dried blood as compared with the influence of the non-legumes. Experiments in which the crop residues alone were concerned leave less room for doubt, the conclusions of the three investigations on that subject being unanimous in favor of the legume. In the following experiment an attempt has been made to deal with the question in a somewhat different manner than has been done in the investigations cited.

PLAN OF THE EXPERIMENT

The experiment involved the leaching of soil on which different crops were growing. To accomplish the leaching while the crops were on the soil and to obviate the necessity of removing and consequently of aerating it, the soil was placed in cylindrical cans with funnel-shaped bottoms from which the drainage water was conducted by pipes connected with large bottles in which the leachings were collected. The cylinders were 12 inches in diameter and 2 feet deep. Ten inches at the bottom were filled with a weighed quantity of clay subsoil and the funnel-shaped bottom was filled with sand and gravel.

It was intended that the soil should be very rich in readily nitrifiable nitrogen, for which purpose dried blood was added to the soil at the rate of 45 gm. per can. Acid phosphate was added at the rate of 15 gm., muriate of potash at the rate of 8 gm., and ground limestone at the rate of 90 gm. per can. The same weight of soil was placed in each can.

The soil used in the experiment was a sand of moderate fertility and good drainage. The commercial fertilizer used in such liberal quantities made it productive and the lime permitted an abundant growth of clover. No difficulty was experienced in leaching the soil and the drainage water was clear.

Twelve cylinders were employed, six of which were planted to timothy and six to red clover. The soil of all cans was inoculated with a pure culture of *Bacillus radicicola* from the clover plant. Each can also received 1000 cc. of an infusion made by stirring 1 pound of garden soil with 3 liters of tap water. This was to insure an active nitrifying flora. The crops were planted on September 15 and on September 29 there was a good stand.

During the time the timothy and clover were growing the soil was leached from time to time, and as water was dripping from the outlets much of the time the soil in all cans contained about the same quantity of moisture. All the water applied had been distilled and was free from nitrates. The growth of the plants was sufficient to permit leaching on November 27, and from that time until the crops were harvested the soil of each can was leached about once a month.

It was intended to estimate the nitrogen contained in the drainage water and all of this proved to be in the form of nitrates which were determined by the phenol disulfonic acid method. There were seven leachings during the time the timothy and clover were on the soil.

NITROGEN IN CROPS

The crops were harvested on May 13. Both crops had made a good growth, the clover relatively better than the timothy, but both were healthy and vigorous. At the time of harvest the clover was in blossom and the timothy had not yet reached that stage. The crop from each can was dried, weighed and its nitrogen content determined. The yields of dry matter and of nitrogen in these crops are shown in table 1.

The total quantity of nitrogen removed from the soil in the cans on which timothy was grown was small as compared with the quantity added in the form of dried blood. This material contained 10.5 per cent of nitrogen and, consequently, at the rate of 45 gm. of dried blood per can the total amount of nitrogen thus added to the six timothy cans would be 28.35 gm. The crop removed a total of 4.0962 gm. from the six cans, thus leaving a large quantity of easily nitrifiable nitrogen remaining in the soil. It is impossible to say how much nitrogen the clover took from the soil.

TABLE 1

Weights of dry matter and nitrogen in timothy and red clover grown in cans 9–20

Can Number	TIMOTHY		Can Number	RED CLOVER	
	Dry matter	Nitrogen		Dry matter	Nitrogen
	gm	*gm*		*gm*	*gm*
9	46 95	0 5978	15	179 86	5 3669
10	60 25	0 7497	16	169 99	5 0688
11	66 65	0 7866	17	168 75	4 9104
12	68 03	0 7840	18	165 27	4 8894
13	49 19	0 5712	19	179 83	5 3865
14	49 09	0 6069	20	170 68	5 1015
Total.....	340 16	4 0962		1034 38	30 7235

TABLE 2

Nitrogen in leachings during growth of timothy and red clover

CAN NUMBER	CROP	NITROGEN IN DRAINAGE WATER BETWEEN DATES INDICATED							TOTAL NITROGEN	TOTAL VOLUME OF DRAINAGE WATER
		Sept 29 to Nov 27	Nov 27 to Dec. 11	Dec 11 to Jan 14	Jan 14 to Feb 11	Feb 11 to Mar 11	Mar 11 to April 11	April 11 to May 11		
		gm	*gm*	*gm.*	*gm*	*gm*	*gm.*	*gm*	*gm*	*liters*
9	Timothy	0 23	0 12	0 14	0 004	0 0005	0 0005	trace	0 4950	28.043
10	Timothy	0 23	0 10	0 08	0 003	trace	trace	trace	0 4130	27 342
11	Timothy	0 22	0 07	0 02	trace	trace	trace	trace	0 3100	23.188
12	Timothy	0 24	0 07	0 03	trace	trace	trace	trace	0 3400	26 431
13	Timothy	0 26	0 13	0 08	0 004	trace	trace	trace	0 4704	28 882
14	Timothy	0 17	0 07	0 06	0 004	trace	trace	trace	0 3004	29 583
Total		1 35	0 56	0 41	0 0078	0 0005	0 0005	trace	2 3288	163 469
15	Red clover	0 17	0 06	0 09	0 008	0 0012	0 0009	trace	0 3301	27 391
16	Red clover	0 14	0 06	0 07	0 019	0 0023	0 0014	0 0016	0 2943	25 720
17	Red clover	0 17	0 06	0 07	0 018	0 0027	0 0011	0 0007	0 3225	28 923
18	Red clover	0 16	0 09	0 08	0 016	0 0025	0 0011	0 0009	0 3505	30 100
19	Red clover	0 13	0 04	0 07	0 016	0 0009	0 0005	0 0007	0 2581	22 535
20	Red clover	0 16	0 04	0 01	0 003	0 0011	0 0005	0 0003	0 2149	18 884
Total.........		0.93	0 35	0 39	0 080	0 0107	0 0055	0 0042	1 7704	153 553

NITROGEN IN DRAINAGE WATER DURING GROWTH OF TIMOTHY AND RED CLOVER

The soil in the cans was leached with distilled water at intervals during the time the timothy and clover were growing. A record was kept of the amount of water that passed through the soil and of the nitrates (NO_3) in parts per million contained in the percolate. Table 2 shows the quantity of nitrogen removed in the drainage water from each can at each leaching, also the total volume of water percolating through the soil during the time the timothy and clover crops were growing.

It may be seen from the figures in the table given above that most of the nitrogen leached out of the timothy soil was in the first two leachings and that the last three leachings contained practically none. Red clover, on the other hand, continued to give appreciable quantities in all the leachings. Apparently nitrification continued more actively in the clover soil than in the timothy soil while the crops were making their later growth, or else the clover made less use of the nitrate nitrogen of the soil than did the timothy. The quantity of nitrate nitrogen leached from the clover soil after growth was well started was so small as to indicate that either nitrification had practically stopped or that the clover was absorbing nitrate nitrogen.

NITROGEN IN DRAINAGE WATER AFTER THE SOIL HAD REMAINED FALLOW FOR A MONTH

The crops having been harvested, the soil was stirred up to a depth of about 2 inches and the stubble and coarse roots near the surface were removed, but the remainder of the roots were left in the soil The soil was allowed to stand in this condition from May 13 to June 15, water being added from time to time, but no vegetation being allowed to develop It was then leached and nitrates were determined in the leachings The volume of drainage water from each can and the nitrogen it contained are stated in table 3.

TABLE 3

Nitrogen in drainage water from fallow soil

CAN NUMBER	PREVIOUS CROP	VOLUME OF DRAINAGE WATER	NITROGEN IN DRAINAGE WATER	CAN NUMBER	PREVIOUS CROP	VOLUME OF DRAINAGE WATER	NITROGEN IN DRAINAGE WATER
		liters	*gm*			*liters*	*gm*
9	Timothy	6 958	0 0156	15	Red clover	7 270	0 1215
10	Timothy	6 158	0 0099	16	Red clover	7 605	0 0927
11	Timothy	7 582	0 0121	17	Red clover	7 250	0 0884
12	Timothy	7 850	0 0120	18	Red clover	8 205	0 1297
13	Timothy	7 060	0 0172	19	Red clover	6 452	0 0933
14	Timothy	7 228	0 0359	20	Red clover	7 445	0 1446
Total		42 836	0 1027	Total .		44 227	0 6702

When the soil was allowed to lie fallow the formation of nitrates was much more active in the soil previously in red clover than in that which had raised timothy. The figures given above indicate a production of about six times as much nitric nitrogen in the clover soil. The timothy soil had a large quantity of easily nitrifiable nitrogen still present in the form of dried blood, as only a small proportion of this had been used by the previous crop or had been leached out. The greater quantity of nitrates leached from the clover soil during this period is evidently not to be accounted for by the assumption that the clover crop did not absorb nitrates. The fact that the soil had been leached several times while the clover was growing and that the quantity so obtained was small, would invalidate this assumption. There was, without doubt, a much more active formation of nitrates following the growth of clover than of timothy.

THE PRODUCTION OF CROPS ON SOILS PREVIOUSLY PLANTED TO TIMOTHY AND TO RED CLOVER

On June 22 four of the cans were planted to oats and four to maize, the remaining four being allowed to continue in fallow and being left free of vegetation during the entire time the other crops were on the soil. Both the oats and maize plants were beginning to appear above ground on June 27. The oats were thinned to 18 plants per can and the maize to 3 plants. Early in July the cans were taken out of the greenhouse and remained outside until the latter part of September, during which time they received the rainfall. The quantity of distilled water added to each can in addition to the rainfall is shown in table 4.

TABLE 4

Volume of water added to each can between June 22 and October 21

CAN NUMBER	PREVIOUS CROP	PRESENT CROP	WATER ADDED	CAN NUMBER	PREVIOUS CROP	PRESENT CROP	WATER ADDED
			liters				*liters*
9	Timothy	None	30	15	Red clover	None	28
10	Timothy	Oats	45	16	Red clover	Oats	54
11	Timothy	Maize	40	17	Red clover	Maize	46
12	Timothy	None	29	18	Red clover	None	27
13	Timothy	Oats	47	19	Red clover	Oats	53
14	Timothy	Maize	39	20	Red clover	Maize	43

It will be seen at once that the oats and maize on the soil previously in red clover required more water than did the same crops on the timothy soil. This was reflected in the yields of crops, which was greater on the clover soil. The fact that the oats required more water than the maize is probably to be accounted for by the small growth of the latter.[1] The fallow soils all received nearly the same quantities of water.

Throughout the entire period of growth the plants on the clover soil were more vigorous. On July 15 maize on timothy soil had 5 leaves and that on clover soil 7 leaves, and the latter was a deeper green and taller. On the same date the oats on the clover soil had tillered nearly twice as much as on the timothy soil and was likewise a better color. Oats, however, did not show as much benefit from the clover at an early stage as did maize.

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[1] The maize was a very small and early maturing variety, which probably accounts for the small production of dry matter and nitrogen in the crop. It would doubtless have been better to have used a somewhat larger variety.

Maize on clover soil began to show some tassels on August 15, that on timothy soil on August 20. Oats on clover soil began to head on August 17 and that on timothy soil on August 21.

On September 23 the maize had ripened, but the oats were only partly yellow. The rather unusual occurrence of maize ripening before oats was probably brought about by the late planting of the oats, which necessitated its early growth being made during warm weather and to the naturally early maturing character of the maize.

TABLE 5

Nitrogen in oats and maize following timothy and red clover

PREVIOUS CROP	CAN NUMBER	NITROGEN IN OATS AND MAIZE
		gm
Timothy......	10, 11, 13, 14	1 3346
Red clover...	16, 17, 19, 20	2 3951

TABLE 6

Volume and analysis of drainage water collected between June 22 and November 14

CAN NUMBER	PREVIOUS CROP	VOLUME OF DRAINAGE WATER	NITROGEN IN DRAINAGE WATER
		liters	*gm*
9, 10, 11, 12, 13, 14	Timothy	89 018	1 4353
15, 16, 17, 18, 19, 20	Red clover	86 793	3 2460

The crops were left on the soil until October 21, at which time there was no question that the maize was fully ripe, but the oats were still partly green. The crops were harvested on this date by cutting off the stems at the surface of the soil, after which the grain and straw or stalks were separated. They were then dried, ground and subjected to analysis, the results of which are shown in table 5.

The much larger absorption of nitrogen by the crops on the clover soil is at once apparent. The excessive application of dried blood to the previous crop might well be expected to have had a residual effect on the crops grown on the timothy soil but this was slight as compared with the influence which red clover exerted on the nitrogen content of the crops which followed that legume.

After the crops were removed the soil of each can was subjected to repeated leachings in order to remove the nitrates as completely as practicable, and on the figures of the last leaching the quantity of nitrates remaining in the water in the soil was calculated. The results of the leachings during the time when the oats and maize were growing and after their removal, together with the nitrates remaining in the soil water, are shown in table 6.

The total quantity of nitrate nitrogen in the drainage water following clover was more than twice as great as in the water following timothy.

REMOVAL OF NITROGEN FROM SOIL FOLLOWING THE GROWTH OF TIMOTHY AND RED CLOVER

It still remains to summarize the total removal of nitrogen from the soil following the growth of timothy and clover. The media in which this nitrogen is contained are (*a*) the drainage water from the soil which lay fallow for a month following the growth of timothy and clover, (*b*) the oat and maize crops raised after the fallow, and (*c*) the drainage water from the soil on which the oats and maize were growing and from the bare soil during the same time. These removals of nitrogen are itemized and totaled in table 7.

TABLE 7

Nitrogen contained in crops and drainage water following timothy and red clover

	CAN NUMBERS	
	9, 10, 11 12 13, 14	15 16, 17 18, 19, 20
Previous crop	Timothy	Red clover
Nitrogen in drainage from fallow soil (gm) .	0 1027	0 6702
Nitrogen in crops of oats and maize (gm).	1 3346	2 3951
Nitrogen in drainage from oats and maize and bare soil (gm)	1 4353	3 2460
Total quantity of nitrogen removed (gm).	2 8726	6 3113

Comparing the total quantities of nitrogen removed from the two soils it will be seen that there was a little more than twice as much from the clover soil as from the timothy soil. The quantity in the drainage from the fallow soil during the period from May 13 to June 15 was six times as great from clover soil as from the timothy soil, while the drainage from the oat, maize and bare soil during the period from June 15 to November 14 contained only a little more than twice as much nitrogen from the clover soil as from the timothy. The effect of the clover in increasing nitrate production apparently began to subside after a few weeks. In the drainage water analyzed on October 21 the nitrogen from the clover soil was only about one and one-half times as much as from the timothy soil. Table 8 shows the quantities of nitrogen removed from the soils kept bare of vegetation from the time the timothy and clover were removed in May until the middle of November.

It is very apparent from this table that the clover soil was losing its nitrate-producing power much more rapidly than was the timothy soil. Nitrate production was nine or ten times as rapid in the clover soil immediately after taking off the clover crop, while six months later it was only about one-fourth greater. The effect of clover in increasing nitrate production is not of long duration under the conditions of this experiment and if it is as short-lived in field soils the practice of not plowing up a clover sod until the farmer is ready to replant the land would seem to be advisable. If, however, the conditions had been less favorable for nitrification, the effect of the clover might have been more enduring. In this case there is some question whether the effect of clover on nitrate formation is greater after the first succeeding grain crop than is the effect of the timothy. It should be noted, however, that the clover was on the soil for only a short time and the soil was spaded up immediately after removing the first crop of hay. It should also be noted that the grain crops in the clover soil were twice as large as those in the timothy soil, which is an unusually marked effect of the clover and is probably to be explained by the fact that available nitrogen was the limiting factor in the growth of the grain crops, all the other plant nutrients having been supplied in excess.

TABLE 8

Relative nitrate-producing capacity of uncropped soil previously growing timothy and red clover

PERIOD BETWEEN LEACHINGS	ACTUAL PRODUCTION		RELATIVE PRODUCTION	
	Timothy, cans 9, 12	Clover, cans 15, 18	Timothy, cans 9, 12	Clover, cans 15, 18
	gm	*gm*	*gm*	*gm.*
May 13–June 15 ...	0 0276	0 2512	100	910
June 15–July 15. ..	0 0199	0 1998	100	1004
July 15–July 26 . .	0 0670	0 1720	100	257
July 26–August 14	0 0883	0 3340	100	387
August 14—September 7 .	0 3113	0 6800	100	218
September 7–September 23	0 2183	0 4452	100	204
September 23–October 21.	0 1363	0 1954	100	143
October 21–November 4	0 1137	0 1769	100	155
November 4–November 14 .	0 1861	0 2284	100	123
Remaining in soil water	0 0419	0 0405		
	1 2104	2 7234		

If the more abundant formation of nitrates in the clover was due to the more easily nitrifiable character of the nitrogenous organic residue of that crop it would seem probable that this material constitutes only a minor part of the total nitrogenous organic matter in the clover roots. At least this is the case if we assume that one-third of the nitrogen of the clover crop remains in the soil. Calculated on this basis about 15 gm. of nitrogen would have remained in the six cans in which clover was grown. There were 3.4387 gm. of nitrogen in the crops and drainage water of the clover soil in excess of that in the timothy soil. Something over one-fifth of the total nitrogenous residue of the clover crop had therefore nitrified by the time that the rate of nitrification in the clover soil had declined to the rate of the timothy soil. The remainder of the residue from the clover would not appear to be much more easily nitrified than the nitrogenous residue from the timothy.

The experiment brings out certain data which may be significant. In the first place it has shown that more abundant formation of nitrates took place in clover soil than in timothy soil even when both soils had received a heavy application of dried blood containing several times as much easily nitrifiable nitrogen as was contained in the crop of timothy removed. It has also demonstrated that under the same conditions the growth of oats and maize varied directly with the quantity of nitrates when these crops were produced under conditions in which nitrogen was presumably the limiting factor. It has, furthermore, shown that only a minor part of the total nitrogen probably remaining in the roots of the clover crop was nitrified before the rate of nitrate production in the clover soil fell to that of the timothy soil.

SUMMARY

Twelve cylinders capable of being leached were filled with a soil of medium fertility and good drainage qualities. The soil was abundantly limed and fertilized with acid phosphate, muriate of potash and dried blood. Six cylinders were planted to timothy and six to red clover. The soil of all cans was inoculated with *B. radicicola* from clover nodules.

During the time the timothy and clover were growing the soil was leached with distilled water from time to time. Nitrogen was determined in the drainage water and in the crops of timothy and clover. After these crops were removed the soil was allowed to remain in fallow for a month, leached and nitrogen determined in the drainage. Of the cylinders on which timothy had been grown two were planted to oats, two to maize and two kept free of vegetation. The clover cylinders were treated in the same way. All were leached from time to time and nitrogen determined in the drainage water and also in the crops.

There was little difference in the quantities of nitrogen leached from the timothy soil and clover soil during the time those two crops were growing on them. There was about six times as much nitrogen leached from the clover soil during the month that both soils stood fallow after the timothy and clover crops had been removed. There was only about twice as much nitrogen leached from the fallow clover soil as from the timothy soil during the next five months. At the end of this period the rate of nitrate production in the clover soil was little greater than in the timothy soil. The crops of oats and maize following clover were larger and contained more nitrogen than did those following timothy.

The experiment taken as a whole shows that under the same conditions of soil and treatment clover caused a greater production of available nitrogen than did timothy. This effect is shown in the nitrate content of the drainage water and the total nitrogen content of the oats and maize. Whether the clover stimulated the nitrification process or whether it contributed easily nitrifiable material is not apparent from the data. If the greater production of nitrates in the clover soil was due to the decomposition of the residue of that crop it appears that a portion of this residue is more easily nitrifiable than dried blood and that it constitutes only a small part of the entire residue of the clover crop.

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SAMPLING SOIL PLOTS

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Methods of sampling the soil of fertility plots for investigation as to its chemical content are by no means standardized. One is often led to question the conclusions drawn by investigators from their analytical results when the method of choosing the samples was not indicated or, when given, showed a lack of appreciation of the necessity of accuracy in their selection.

The Official Methods of the Association of Agricultural Chemists (4) leave much to be desired in specific suggestions concerning this matter. The directions specify the taking of a "composite sample" with no statement as to the number or location of the individual parts of the composite.

Wiley (10) reviews the methods employed by the more prominent agricultural experiment stations of Europe. In most cases holes are dug with a spade and the sample is secured by making a composite from slices taken from the sides of these holes. The location or number of these holes is not specified.

Warington (8) points out the difficulties involved in sampling the same plot after some years due to the fact that the quantity of organic matter may be changed and the same volume of soil may not represent the same weight of inorganic matter as was contained in the original sample. He also notes that samples should be chosen for comparison when they are in the same condition, i.e., following the same crop and treatment as to cultivation, and suggests securing the sample after a crop has been harvested and before plowing. Warington gives the method adopted by the Rothamsted station and followed since 1865. In this method a square frame of sheet-iron covering an area of 144 square inches is driven into the soil to a depth of 9 inches and the soil contained within this area constitutes the sample.

Many of the methods given are intended for soil survey purposes. The method employed by the United States Bureau of Soils is given by Whitney (9). Hopkins (2) adopted the very definite plan of taking samples to uniform depths but gives no specifications as to distribution of borings in selecting a composite. It is probably not the intention of any of those engaged in soil survey to return to the identical areas from which the samples were chosen, to collect a second sample at some future date for purposes of comparison. Nevertheless, there are on record numerous cases in which conclusions have been drawn from samples chosen at different dates from approximately the same area, in which no more adequate precautions were taken than those indicated above for insuring that they actually represented the areas in question.

Unless it is possible to sample a given area of soil as many times as may be desired on the same day with the result that the samples are practically identical in composition, there is no reason to believe that conclusions drawn from a comparison of analyses of samples chosen from the same areas at periods several years apart will be trustworthy. This is particularly true if the work is done by different men whose ideas as to how to sample a plot of ground differ.

Leather (3) chose duplicate samples made up of a composite, of 12 borings each, to a depth of 9 inches from areas on three Indian experiment stations. Determinations of the content of nitrogen, phosphoric acid and potash were made. The laboratory error was least on the nitrogen. The duplicate samples varied from 0.001 to 0.015 per cent of this element, with an average variation of 0.006 per cent.

Thiel (7) selected an area of 200 square meters of uniform soil in which, after the soil had been spaded to plow depth, 10 stakes were driven as locations for sampling. A spadeful of soil was selected from near each stake. The composite sample was then passed through a 2-mm. sieve, thoroughly mixed and dried. These operations were repeated twice each month for a year. Five determinations were made on each composite with fairly concordant results. The nitrogen content of the bimonthly composites, calculated to a depth of 50 cm., varied from 9,090 to 10,269 kgm. per hektare.

Pfeiffer and Blanck (5) chose five samples of soil from each of 6 plots located on an area of 9 square meters of land. Four holes were dug on each plot to a depth of 25 cm., and a slice was taken from the side of each hole for the composite. Determinations were made of the nitrogen in the composites. In no case did the nitrogen in any composite vary from the mean of the 5 composites of that plot by more than 0.0063 per cent.

Robinson and Lloyd (6) determined the experimental error in sampling soil in two fields, one of which was quite uniform in texture and the other was not. We will confine the discussion to the uniform field. No records are given as to depth of sampling or type of auger used. Fifteen borings were made at equi-distant points from an area of ¾ acre. Each boring was analyzed separately for its content of phosphoric acid. Assuming that the weight of each boring was the same, one can make certain calculations as to what the percentage of phosphoric acid would have been with any combination of samples of a composite. The results of these calculations show that the composite made up of the entire 15 borings would have contained 0.219 per cent of phosphoric acid. By selecting other composites of 5, 6, 7, 9 and 11 borings chosen according to regular schemes over the area, the percentage of phosphoric acid reported might have varied from 0.212 to 0.229. The analytic error is shown in this investigation, however, to be greater than the sampling error.

Bear and Salter (1) chose 6 composites, each consisting of 38 borings, to a depth of 6⅔ inches, from a plot of 1/10 acre. These borings were distributed at regular intervals over the plot. The soil was passed through a 2-mm. sieve and then pulverized to pass a 100-mesh sieve. Each composite was analyzed for total carbon, nitrogen and phosphorus. Five of the six composites were quite uniform in their content of these three elements. The greater variation in the total carbon was 0.07 per cent, in total nitrogen 0.009 per cent, and in total phosphorus 0.002 per cent. In the sixth composite the content of these three elements was higher than the lowest of the five other composites by 0.15 per cent of carbon, 0.018 per cent of nitrogen and 0.004 per cent of phosphorus. The greatest variation in duplicates of any composite was 0.02 per cent of carbon, 0.001 per cent of nitrogen and 0.001 per cent of phosphorus.

OBJECT OF THIS EXPERIMENT

It seems desirable to have some very definite plan of selecting soil samples from fertility plots if analytical comparisons are to be made from time to time of the effect of the fertilizer or other treatments the plots receive. It is logical to decide on the method to be employed before field work is begun in order that samples of the original soil may be selected and set aside under appropriate conditions for comparison with samples to be chosen subsequently from the same plot.

The first question which it was thought best to investigate was that of determining how to choose a composite which would accurately represent a plot for the particular day on which it was chosen. No consideration therefore, was given to the question of the relationship of this sample to one which might be chosen from the same area 10 to 20 years hence.

PLAN OF THIS EXPERIMENT

Two plots of ground each 1/20 acre in size (21 ft. by 103.7 ft.) were set aside for this test. Plot I was as uniform as could be secured on the experimental field. Plot II was chosen for its lack of uniformity. Stakes were driven at various points in the plots and strings were stretched from stake to stake. At points of intersection samples were chosen. This located the samples at the 45 points indicated in figure 1 The samples were chosen with a 1¼-inch auger. The surface samples represented a depth of 6⅔ inches and the subsurface from 6⅔ to 13⅓ inches. Care was taken to have the soil at a moisture content which would permit of removing the auger from the hole without losing any of the core. In this latitude the best time for sampling is usually in the month of November. Surface material, leaves and grass were carefully removed before sampling was begun. Both depths were secured by sinking the auger to the proper depth before removing it with the core. If any surface material chanced to fall into the hole before the selection of the second depth,

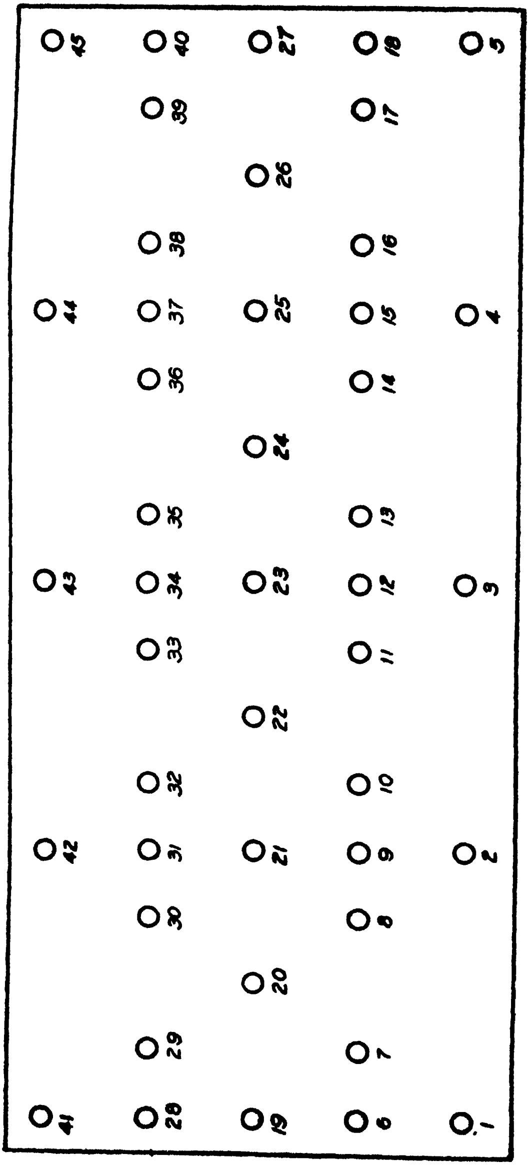


FIG. 1. LOCATION OF BORINGS OVER PLOT

this material was carefully removed with the auger before taking the sub-surface sample. Each boring was placed in a separate container, taken to the laboratory and placed in the drying room. Twenty days later both plots were sampled again in the same manner and within an inch of the same points, the original stakes having never been removed.

Each sample was weighed and prepared for analysis by discarding all material which would not pass a 10-mesh sieve and pulverizing the remainder in a planetary mill to pass a 100-mesh sieve. The weight of the discard was recorded. Nitrogen determinations in 10-gm. duplicates were made of each air-dry sample, making a total for the two samplings of 720 determinations. If the duplicates did not check within 0.1 cc. of $\frac{1}{14}$ *N* alkali they were both discarded and another set was run. This was necessary in only a relatively few cases—the analyst, Mr. J. R Royston, whose services are acknowledged and appreciated, having had considerable experience in nitrogen determinations in soils.

TABLE 1

Arrangement of borings for composites

COMPOSITE	BORINGS
1	1 to 45, inclusive
2	1, 3, 5, 7, 11, 13, 17, 20, 22, 24, 26, 30, 32, 36, 38, 42, 44
3	2, 4, 8, 10, 14, 16, 20, 22, 24, 26, 29, 33, 35, 39, 41, 43, 45
4	1, 2, 3, 4, 5, 19, 20, 21, 22, 23, 24, 25, 26, 27, 41, 42, 43, 44, 45
5	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40
6	1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 19, 21, 23, 25, 27, 28, 31, 34, 37, 40, 41, 42, 43, 44, 45
7	1, 2, 3, 4, 5, 19, 21, 23, 25, 27, 41, 42, 43, 44, 45
8	1, 2, 3, 4, 5, 20, 22, 24, 25, 26, 41, 42, 43, 44, 45

Plot I was not as uniform in composition as had been anticipated. In the first sampling the nitrogen content of the borings varied from 0.155 to 0.258 per cent in the surface soil and from 0.118 to 0.243 per cent in the sub-surface soil. The nitrogen percentages varied in the second sampling in much the same way, but the highest and the lowest nitrogen content were not always found at the same locations on the plot. In the non-uniform plot the nitrogen content of the individual borings fluctuated from 0.113 to 0.226 per cent and that of the sub-surface from 0.097 to 0.236 per cent, with similar variations in the second sampling. Since individual determinations of both weight of sample and nitrogen content were made, it was possible to calculate what the reported analysis would have been if any given combination of borings had been mixed for a composite sample before analyses were made. The quantity of soil in a boring varied considerably. In the first sampling of plot I the largest core contained 248 gm. and the smallest 116 gm.

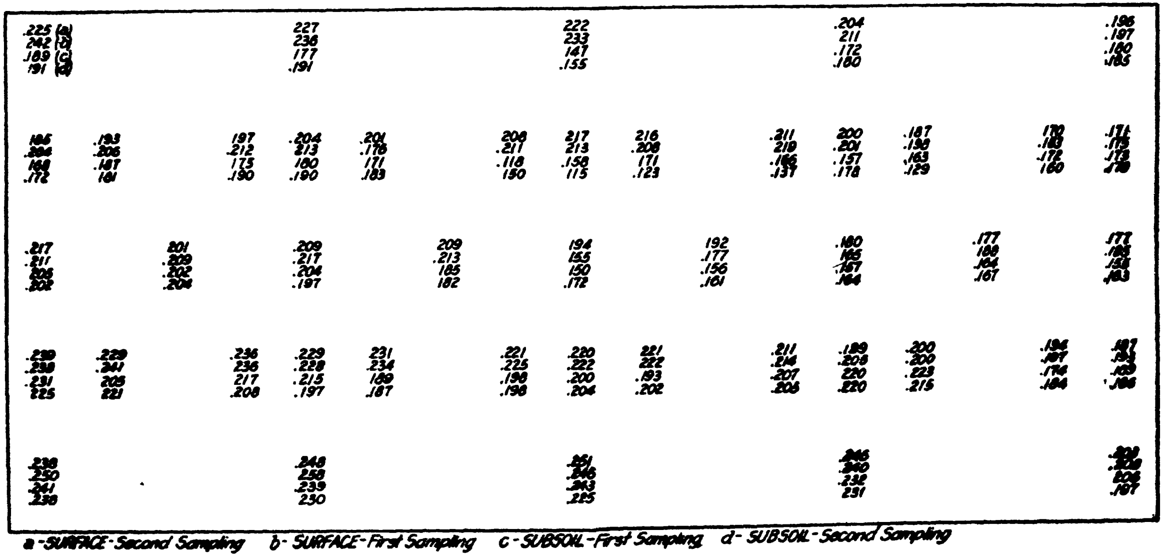
Arranging the single borings into eight groups as shown in table 1 according to what might be considered fairly logical schemes of sampling, it was found that the analyses which would have been reported for any plot on the basis of these composites varied considerably both with the method of choosing the samples for the composite and the number of samples entering into the composite. Table 2 shows the results of this grouping of samples.

TABLE 2

Nitrogen in composites

	COMPOSITE	FIRST SAMPLING	SECOND SAMPLING	DIFFERENCE
		per cent	*per cent*	*per cent*
Plot I. 1st depth	1	0 210	0 210	0 000
	2	0.213	0 210	0 003
	3	0 213	0.212	0 001
	4	0.211	0 213	0 002
	5	0 210	0 207	0.003
	6	0 213	0.212	0 001
	7	0 215	0 217	0 002
	8	0 221	0 219	0 002
Plot I. Second depth	1	0 187	0 187	0 000
	2	0 188	0 186	0 002
	3	0 187	0 187	0 000
	4	0 189	0 192	0 003
	5	0 186	0 184	0 002
	6	0.191	0.192	0 001
	7	0 193	0 193	0 000
	8	0 195	0 196	0 001
Plot II. First depth . . .	1	0 166	0 161	0 005
	2	0 168	0 166	0 002
	3	0 168	0 165	0 003
	4	0 173	0 173	0 000
	5	0 161	0 159	0 002
	6	0 168	0 168	0 000
	7	0 174	0 175	0 001
	8	0 175	0 174	0 001
Plot II. Second depth	1	0 144	0.142	0 002
	2	0 144	0 143	0 001
	3	0 146	0 142	0 004
	4	0 148	0 149	0 001
	5	0 141	0 141	0 000
	6	0 147	0 144	0 003
	7	0 150	0.144	0 006
	8	0 150	0 149	0 001

The outstanding feature brought out by this table is that the nitrogen contents of the duplicate composites are quite uniform, no matter how these composites were selected. This indicates that, within certain limits, the number of samples entering into the composite is not so important as that



a - SURFACE - Second Sampling   b - SURFACE - First Sampling   c - SUBSOIL - First Sampling   d - SUBSOIL - Second Sampling

FIG. 2. NITROGEN CONTENT OF SOIL AND SUBSOIL SAMPLES CHOSEN FROM PLOT 1

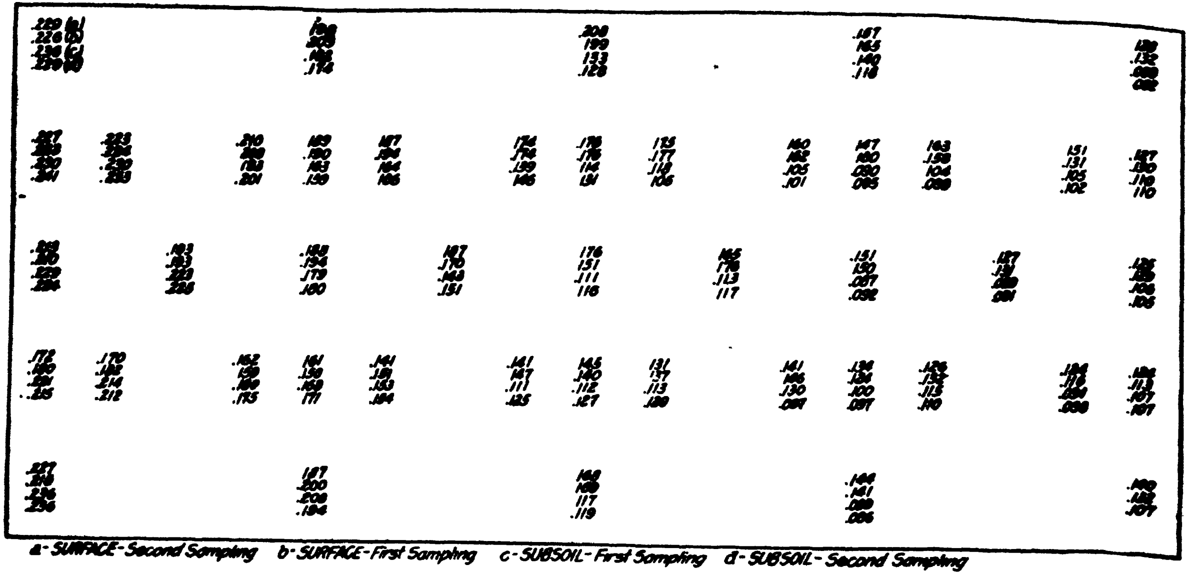


FIG. 3. NITROGEN CONTENT OF SOIL AND SUBSOIL SAMPLES CHOSEN FROM PLOT 2

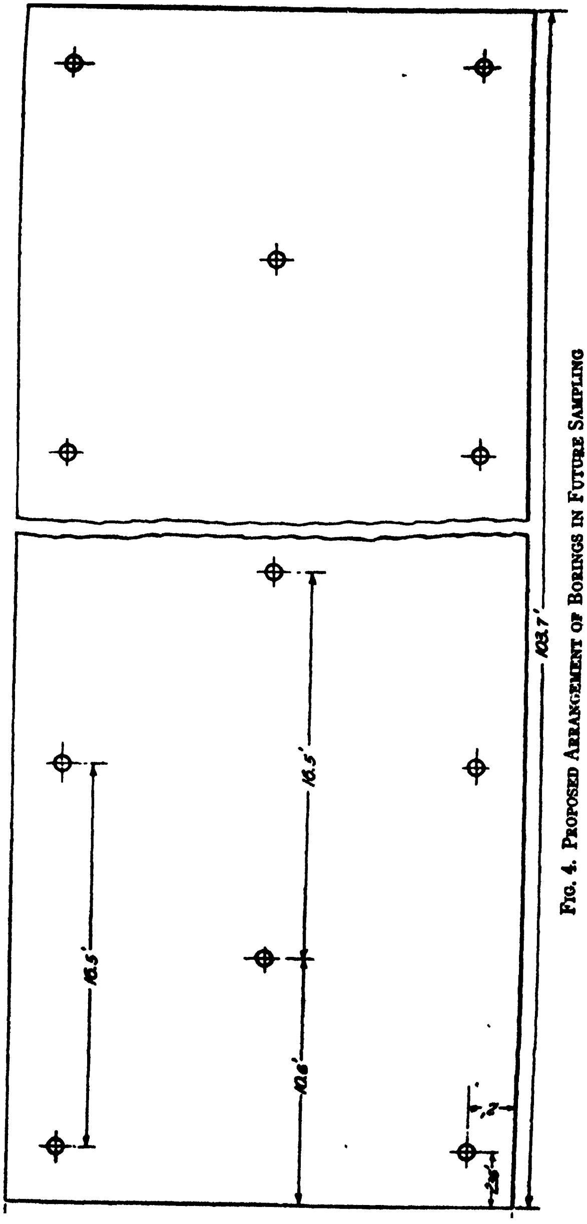


FIG. 4. PROPOSED ARRANGEMENT OF BORINGS IN FUTURE SAMPLING

the samples chosen at different times to represent a plot should be selected from the same locations on the plot. With the more uniform plot the greatest variation was 0.003 per cent, equivalent to 60 pounds of nitrogen per 2,000,000 pounds of soil. With the less uniform plot the greatest variation amounted to 120 pounds per 2,000,000. The composites resulting from the eight groupings of the samples show differences in the nitrogen content of as much as 0.009 per cent, or 180 pounds per 2,000,000.

Reference to figures 2 and 3 shows that it is unsafe to have the composite made up of too few borings. In many cases the cores selected from the same location are almost identical in nitrogen content. At one point in plot 1, however, the variation amounted to 0.039 per cent and at several other points it exceeded 0.01 per cent. It is possible to so select the borings as to show very marked differences in nitrogen content in duplicate composites, provided the number of borings in the composite is not large.

It would appear from a study of these data that selecting the samples according to the arrangement in figures 2 or 3 or a combination of these two could give results very comparable with those secured from taking the entire 45 for the composite. The total number of borings for the composite would then be 17 or in the latter case 30. If the second samples were chosen from the same locations the data would indicate an almost exact duplication of analysis. It would seem logical, however, to arrange these locations uniformly over the plot. On the basis of these analyses it is safe tc assume that a composite of 20 borings distributed uniformly over the plot is satisfactory, provided in subsequent sampling the locations of the borings are practically identical with those of the first sampling. The effect of cultural treatment with several years intervening will be left for a subsequent report.

CONCLUSIONS

On the basis of this study it seemed desirable to locate the samples of soil for the composite representing each plot according to the arrangement indicated in figure 4. There appears to be no argument in favor of selecting separate samples of the surface and sub-surface soil, but it does seem desirable that the samples be chosen to a depth well beyond the plow line. According to this plan the composite from each $\frac{1}{20}$-acre plot would be made up of 20 samples, each chosen to a depth of 12 inches and arranged over the plot as indicated in the diagram. In all subsequent sampling of these plots the location of the samples and the method of choosing them should be as nearly an exact duplication of the first sampling as possible.

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THE EFFECT OF AERATION AND OTHER FACTORS ON THE LIME REQUIREMENT OF A MUCK SOIL

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INTRODUCTION

After the work reported in this paper had been practically completed the attention of the author was called to a much more elaborate investigation by Conner (1) along the same line. In view of Conner's paper it might almost seem that the present one is superfluous. However, the methods and conditions of the two investigations differed sufficiently to justify publication of the results of both as supplemental to each other.

METHODS USED AND EXPERIMENTAL RESULTS

In April, 1919, the writer had occasion to determine the lime requirement of some muck soil from the shore of Lake Pontchartrain, Louisiana, and was impressed by the fact that the long time required for air-drying, together with the frequent stirring, probably brought about chemical changes which would affect the determination. Such oxidation changes would presumably reduce the acidity. To test this point some of the soil was air-dried in the usual way and ground to pass a 2-mm. sieve. Another portion was kept in the original moist condition in a sealed jar. Moisture was then determined in both the air-dried and the moist portions and the lime requirement of each was determined by the Johnson method (2). The equivalent of 10 gm. of dry soil was weighed in each case and enough water added to the air-dried soil and to the blanks to make the total amount of water the same in all cases. The mixtures of soil and bicarbonate solution and also the blanks were shaken 10 minutes before filtering.

It is recognized that the Johnson method for lime requirement is rather new and has not yet been generally adopted. However, it is rapid, simple, and concise in its results, and is believed to be eminently suited to an investigation like the present one where comparative results only are desired.

Contrary to expectation, the air-dried sample gave a higher result than the undried sample. In other words, it appeared that aeration had increased the acidity.

The question then arose as to whether a drained and cultivated muck soil would actually tend to become more sour than if it were left undisturbed in its original overflowed, water-logged condition. Incidentally, it was desired to know how much effect lime, in the quantity indicated by the Johnson method, would have in reducing acidity through a considerable period of time, and what changes might take place during storage of the moist and air-dried soil, respectively.

TABLE 1

Lime requirement of muck soil after various treatments

(Expressed in per cent of calcium carbonate on the basis of oven-dry soil)

TREATMENT		APRIL			DECEMBER (COMPARE WITH APRIL)	DECEMBER		
		Moisture	Lime requirement		Lime requirement	Moisture	Lime requirement	
		per cent	*per cent*		*per cent*	*per cent*	*per cent*	
A. None....		76 4	0 501 0.471	0 486	0.615	76.1	0.540	
B. Air-drying.............		19.1	0.559 0 536	0 547	0.418	18.3	0.456	
C. Aerobic....	1					60.7	0.489	0.501
	2					66.6	0.514	
D. Anaerobic...........	1					78.6	0.598	0.608
	2					83.1	0 617	
E. Lime plus aerobic....	1					63.7	0.411	0.414
	2					58.9	0.418	
F. Lime plus anaerobic.	1					85.0	0 553	0.543
	2					85 2	0.534	

Different portions of the soil were lettered and preserved as follows:

A. The original moist soil (76.4 per cent moisture), sealed in a glass jar.

B. Same as A, only air-dried and ground to pass a 2-mm. sieve. Kept in an ordinary screw-top sample jar.

C. Same soil as A; 150 gm. in each of two glass jars, 1 and 2. Jars kept uncovered. Soil kept moistened with distilled water and stirred occasionally.

D. Same soil as A; 150 gm. in each of two glass jars, 1 and 2. Soil covered with distilled water and jars kept tightly sealed.

E. Same as C except for addition of calcium carbonate in the amount indicated by the original determination on A.

F. Same as D except for the addition of calcium carbonate as in E.

The tests were started in April, 1919, and continued approximately 8 months. At the end of the period (December, 1919) the contents of jars D and F were transferred to large porcelain dishes and exposed to the air, with occasional stirring, for a few days until the excess of water had evaporated. Moisture was then determined in all of the samples, including the original A and B, and the lime requirement was determined by the Johnson method, distilled water being added to all samples (except the wettest one—F2) and to the blanks in quantity to make the relation of dry soil to total liquid the same in all cases.

Also, determinations were made on A and B using the same quantity of dry soil and total liquid as was used for the original determinations on these samples in April, in order to afford direct comparison between the two dates.

The results obtained, expressed in percentage of calcium carbonate required to neutralize the oven-dry soil, are shown in table 1.

DISCUSSION OF RESULTS

The figures given in table 1 plainly indicate that more acidity was developed under water-soaked, anaerobic conditions than when the moist soil was kept aerated by stirring. Comparing C with D and E with F it will be seen that the lime requirement of the anaerobic soil exceeds that of the aerobic soil by more than 0.1 per cent in each case. However, in view of Conner's results (1) showing that the acidity developed by a peat soil is greater the more water it contains, it cannot be considered as proved that aeration was the controlling factor in the experiments here reported. D and F were much wetter than C and E, respectively, and therefore would probably have developed more acidity even if C and E had not been mechanically aerated. But of course the logical interpretation of Conner's results, as he himself recognizes, is that the drier soils are better aerated by diffusion than are the wetter soils, and that the oxidation thus accomplished is at least one factor which tends to decrease acidity.

If the Johnson method furnishes a true or even approximate measure of soil acidity, then it appears that the addition of lime to this soil has greatly increased the rate of formation of acids. Thus sample E, which was supposed to be exactly neutral at the beginning of the experiment, developed during the 8 months a lime requirement of 0.414 per cent, while sample C, which started with a requirement of 0.486 per cent, showed 0.501 per cent at the finish—or a difference of only 0.015 per cent developed during the 8-month period. Similarly, sample F, which was neutralized with lime at the beginning, developed an acidity of 0.543 per cent, while sample D, without lime, developed only the difference between 0.608 and 0.486 per cent, or 0.122 per cent. This accelerated rate of acid formation is probably not to be explained by the presence of the calcium, but by the Law of Mass Action. The exactly neutralized soil would naturally develop acidity much more rapidly than one already containing much acid.

However this may be, it is evident that in liming a muck soil for permanent results one should apply much more lime than is indicated by the lime-requirement determination, in order to take care of subsequently developed acidity.

As already noted in the first part of the paper, sample B, air-dried, showed a higher lime requirement than sample A, undried. Conner also found that air-drying increased the acidity in many cases, though not in the case of an organic soil containing much water, such as sample A.

But when samples A and B were kept in storage for 8 months some notable changes took place. Sample A, containing 76 per cent of water, developed a considerable increase of acidity (changing from 0.486 to 0.615 per cent) while the air-dried sample (19 per cent moisture) changed in the other direction, from 0.547 to 0.418 per cent.

Some light is thrown on the nature of the Johnson method for lime requirement, and all similar methods, by a consideration of the two sets of values found for A and B in December. In the first case, where the same ratio of dry soil to total liquid was used as had been used in the April determinations, somewhat different results were obtained than in the second case where a greater amount of total liquid was used in order to make the results comparable with those of C, D, E and F. This, of course, merely emphasizes the well known fact that all such methods are only comparative, not absolute, and that conditions must be rigidly controlled in using them.

SUMMARY

A typical black muck soil was treated in various ways in order to study the effect of aeration and other factors on lime requirement as determined by the Johnson method.

It was found:

That the lime requirement of this soil was increased by air-drying.

That when the soil was kept moist and frequently stirred for a period of 8 months the increase of acidity was decidedly less than when it was kept covered with water in a sealed jar.

That soil which was first neutralized with calcium carbonate and then kept for a period of 8 months, under either aerobic or anaerobic conditions, developed a great deal more acidity than did unneutralized soil under the same conditions. This is explained by the Law of Mass Action.

That the lime requirement of all samples stored in a moist condition was increased, but that the air-dried sample decreased in acidity during storage.

It was emphasized that results obtained by the Johnson and similar methods are comparative rather than absolute, and that rigid conditions must be adhered to in using such methods.

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THE LIME FACTOR IN PERMANENT SOIL IMPROVEMENT

I. Rotations Without Legumes

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The fact that lime in one or another of its forms has a distinct value in the production of many crops, and in improving the physical character of certain types of soil, which in the end means improved crops, has long been known. Thus for centuries lime materials have been used in almost every country and for practically all crops. Various writers have laid special emphasis on the natural fertility of limestone soils, that is, soils having a high percentage of carbonate of lime; and the expression that "a lime country is a rich country" has come to be rather widely accepted. Very much has been written concerning the reasons for the beneficial effects of lime on crop production, but it is probably safe to say that, in the majority of cases, its use has been on general principles rather than as a result of the careful searching out of the reasons for its effectiveness.

It is well known that its use affects some crops more than others, and that some soils are more responsive than others.

Anyone who is at all familiar with the subject has heard the expression, "Lime makes the father rich and the son poor," the meaning of which, no doubt, was that lime aids in "mining" the soil rather than in permanently building it up.

We now know that the words quoted do not necessarily express the facts. Much depends on how the lime is used, and the system of cropping followed, as to whether the son will be poorer or richer than the father.

A quarter of a century ago, little was known as to the influence of lime on soil organisms, whereas we now know that it plays a very important part in this respect.

It has been shown, for example, that there were many more nodules on the roots of soybeans grown on limed plots than on unlimed plots (4, 5), and that both the beans and stalks from limed plots were richer in nitrogen than those from unlimed plots. This perhaps may not be held to be positive proof that lime favors the development of the symbiotic organisms, and that these organisms in turn enable the plant to store up more nitrogen than plants on the unlimed plot; but it is about as near the positive proof as we can come with our present state of knowledge. Certainly, for the practical man, the proof is sufficient.

It has been shown also that, for a period of years, the percentage of nitrogen was higher in alfalfa hay from limed than from unlimed plots, and that, for this period of years, far more total nitrogen was secured in the hay from the former than from the latter (3). Here again, we are lacking the positive direct proof of the beneficial influence of lime on symbiotic organisms, but the indirect proof is at least something to hold to until more direct proof can be secured.

It is now well known that organic matter in the soil, such as crop residues and farm manures, is converted into new plant-food more rapidly on soils that are well supplied with carbonate of lime than on soils that have an acid nature. This is explained on the ground that the organisms which cause the breaking down of the organic matter, with the consequent formation of available nitrogen compounds, find the soil containing the lime carbonate, a more favorable medium for work than the acid soil. Thus it comes about that, other limiting factors being overcome, larger crops, or crops containing a higher percentage of nitrogen, or both, are often produced on soils that are abundantly supplied with the carbonate of lime.

It is conceivable, however, that this process may be carried too far; that is, the breaking down of the organic matter may become excessive, with the so-called "burning out" effect. Indeed, it is very evident that, with favorable conditions and a limited supply of organic matter, this must finally happen. In other words, it is clear that there may be soils—especially those of light, open character—having such a limited supply of organic matter as to require the checking influence of a slightly acid condition.

It is quite evident, therefore, from what has been said, that in considering the lime question, very careful attention should be given to the type of soil, the crops to be grown, and the disposition that is to be made of these crops.

It is quite certain that such experiments, to be of value, should be carried on over a considerable period of years, since the composition of the soil as determined by methods of analysis now in use, is seldom very much changed in a shorter period than 5 years.

It is with the hope of bringing into clearer light the reasons for the use of lime in agriculture and especially in its relation to the permanent improvement of soils, that this work, covering a period of 10 years, is reported.

PLAN OF EXPERIMENT

In 1908 a field experiment was laid out having as its object a study of the nitrogen problem in crop production. The plan includes a study of the sources of nitrogen, the proper amount to apply, the influence of nitrogen on the yield and composition of the crop, denitrification and other nitrogen losses; also, on account of the important bearing which the reaction of the soil has on the nitrogen problem, the plan was made to include the use of lime in the carbonate form. For this purpose, two series of plots were laid out with identical fertilizer treatment—but differing in that the 20 plots hereafter designated as 1A to 20A, were to remain unlimed while the 20 hereafter designated as 1B to 20B were to be limed at stated intervals.

The soil is a Sassafras loam, inclining to a sandy loam, which originally contained 0.11 per cent of nitrogen and 1.22 per cent of total carbon. Lime-requirement determinations were not made at the time the experiment was started, but if we may judge from determinations made on unlimed plots later, it is safe to assume that the lime requirement in 1908 was about 1600 to 2000 pounds of lime (CaO) for 2,000,000 pounds of the soil. As this land had not been under cultivation for a number of years, it is certain that up to 1908, no lime had been applied within recent years.

The plots were laid out $\frac{1}{20}$ acre in size, being about twice as long as wide. The crop rotation, as orginally planned, was: 1 year of corn, 2 years of oats, a year of wheat and one of timothy. This was later changed so that there was 1 year of oats and two of timothy. This is a rotation that was practised more or less some years ago, but would perhaps not be applicable to present conditions.

Since the experiment provides for a study of nitrogenous fertilizer materials, no leguminous crop can be introduced in the rotation, either as one of the main crops or as a green-manure crop. However, it may be noted here that during the summer and fall of 1912, much volunteer clover appeared in the timothy and made considerable growth after the latter had been cut. This clover was later dug out, dried and weighed, and analyzed for nitrogen. The yield of dry matter (clover) from the limed plots was almost double that from the unlimed plots (2). Likewise, samples from the limed plots showed a higher percentage of nitrogen than samples from the unlimed plots.

The lime treatment has consisted of finely ground limestone or oyster shells at the rate of 1 ton per acre just before planting the corn in 1908, and 2 tons per acre again preceding the corn in 1913, the liming thus taking place at intervals of 5 years. A careful record has been kept of the amount of nitrogen applied in the form of manure and fertilizer, and likewise of the amount removed by the crops. Samples of soil were collected for analysis previous to applying the lime in 1913, and again in the fall of 1917.

The manure and fertilizer treatment given these plots, the lime requirement (Veitch method) and the analytical data secured on the soil samples taken at the end of each 5-year period are shown in table 1.

From this table it will be noted that the average lime requirement in 1913 for all of the A plots (unlimed) was 1840 pounds of CaO per acre, and for all the B plots (limed) 1140 pounds. Thus notwithstanding the ton of limestone per acre which was used in 1908, all of the B plots had become quite acid at the end of the first 5 years.

At the end of the second 5 years the average lime requirement for the A plots was 1615 pounds per acre, which is slightly less than the requirement at the end of the first 5 years, although no lime had been applied. On the other hand, the soil of the B plots which had in 1913 received 2 tons of ground

TABLE 1

Lime requirement (Veitch method), total carbon and total nitrogen, 1908–1917

NUMBER	FERTILIZER TREATMENT ($\frac{1}{20}$ ACRE PLOTS)	A'S UNLIMED						B'S LIMED					
		Lime requirement per acre		Total carbon		Total nitrogen		Lime requirement per acre		Total carbon		Total nitrogen	
		First 5 years	Second 5 years	First 5 years	Second 5 years	First 5 years	Second 5 years	First 5 years	Second 5 years	First 5 years	Second 5 years	First 5 years	Second 5 years
		lbs	*lbs*	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*per cent*	*per cent*	*per cent*	*per cent*
1	Nothing . . .	1400	1400	1.39	1.33	0.1059	0.1033	1200	Alk.	1.06	0.90	0.0862	0.0770
2	16 pounds muriate of potash	2100	1600	1.44	1.33	0.1098	0.1173	1000	Alk.	1.14	0.96	0.0871	0.0790
3	32 pounds acid phosphate . . .	2100	1600	1.28	1.24	0.1042	0.1139	1000	200	1.07	0.86	0.0821	0.0751
4	Minerals only* . .	2100	2000	1.36	1.26	0.1180	0.1088	1100	400	1.15	1.04	0.0855	0.0779
5	Minerals and 1600 pounds cow manure	2100	1800	1.38	1.44	0.1002	0.1185	1200	Alk.	1.44	1.42	0.1030	0.1143
6	Minerals and 1600 pounds horse manure .	2200	2700	1.39	1.57	0.0929	0.1230	1500	600	1.43	1.48	0.1028	0.1190
7	Nothing	1600	1000	0.95	0.93	0.0790	0.0785	1000	Alk.	1.17	1.02	0.0879	0.0821
8	Minerals and 8 pounds $NaNO_3$ . . .	1400	1600	1.11	1.03	0.0880	0.0883	1100	200	1.08	1.01	0.0801	0.0787
9	Minerals and 16 pounds $NaNO_3$	2100	1000	1.15	1.13	0.0921	0.0975	1000	200	1.08	0.96	0.0753	0.0810
10	Minerals and $Ca(NO_3)_2$ equivalent to 16 pounds $NaNO_3$.	2100	1000	1.14	1.10	0.0941	0.0903	1200	Alk.	1.12	1.01	0.0805	0.0779
11	Minerals and $(NH_4)_2SO_4$ equivalent to 16 pounds $NaNO_3$.	2100	2000	1.14	1.21	0.0977	0.0904	1500	1000	1.02	1.06	0.0801	0.0819
12	Minerals and $CaCN_2$ equivalent to 16 pounds $NaNO_3$	1400	1000	1.03	1.02	0.0869	0.0844	1000	200	1.16	1.07	0.0861	0.0896
13	Minerals and dried blood equivalent to 16 pounds $NaNO_3$.	2100	1800	1.14	1.23	0.0925	0.1063	1100	600	1.05	0.98	0.0807	0.0826
14	Minerals and dried fish equivalent to 16 pounds $NaNO_3$.	2100	1800	1.26	1.22	0.1014	0.1058	1100	400	1.08	0.93	0.0779	0.0889
15	Minerals and tankage equivalent to 16 pounds $NaNO_3$	1800	1600	1.21	1.18	0.0884	0.0919	1200	Alk.	0.93	0.89	0.0717	0.0803
16	Minerals and 200 pounds alfalfa hay (cut fine)	2100	1600	1.20	1.14	0.0891	0.1012	1100	1000	1.18	1.10	0.0897	0.0904
17	Minerals and 200 pounds rye straw (cut fine). . .	1600	1600	1.26	1.08	0.0950	0.0980	1100	Alk.	1.25	1.15	0.0953	0.0952
18	Minerals and 1600 pounds cow manure plus 16 pounds $NaNO_3$.	1600	2000	1.47	1.36	0.1088	0.1171	1200	Alk.	1.41	1.48	0.1014	0.1190
19	Minerals only	1400	1600	1.29	1.01	0.0980	0.0872	1100	Alk.	1.11	0.95	0.0809	0.0784
20	Minerals and 200 pounds rye straw plus 16 pounds $NaNO_3$.	1400	1600	1.23	1.03	0.0959	0.0908	1100	Alk.	1.14	1.12	0.0832	0.0903
Average.		1840	1615	1.24	1.19	0.0969	0.1006	1140	240	1.15	1.07	0.0859	0.0879

* 32 pounds acid phosphate and 16 pounds muriate of potash.

limestone per acre showed either an alkaline or only a slight acid condition in 1917.

No satisfactory explanation appears for the less acid condition of the A plots in 1917 than in 1913. It is conceivable that such a change might be brought about by the oxidation of organic acids resulting from careful and thorough cultural methods.

Of more interest, perhaps, is a comparison of the percentage of nitrogen and carbon in the soil from the two series of plots. The results are shown in table 1 along with the lime-requirement determinations. A study of the averages shows that there is distinctly less of both carbon and nitrogen in the soils of the limed series than in those of the unlimed series. These averages represent 20 plots in each series, and cover a period of 10 years. The fertilizer treatment which the plots have received is indicated in the table. The majority of the plots have received applications of nitrogenous fertilizers somewhat in excess of general practice, a few have received excessive amounts, while a few others have received no nitrogen at all.

TABLE 2

Average percentage of total nitrogen and carbon in limed and unlimed series at different times

	TOTAL CARBON		TOTAL NITROGEN	
	Unlimed	Limed	Unlimed	Limed
	per cent	*per cent*	*per cent*	*per cent*
Analyses made in 1909 . . .	1.22	1.22	0.1118	0.1118
Analyses at end of first 5-year period (1908-1912)	1.24	1.15	0.0969	0.0859
Analyses at end of second 5-year period (1913-1917)	1.19	1.07	0.1005	0.0879

The results of the nitrogen and carbon determinations seem to confirm a very widespread popular view which holds that lime tends to "burn out" the organic matter of the soil. In the popular mind, however, the "burning out" process is not well understood. It should be thought of as the result of biological processes in the main, rather than of chemical processes.

Moreover, it should be noted that in this case it was the carbonate of lime and not caustic or unslaked lime that was used. It would appear that the carbonate of lime made the conditions more favorable for the development of the organisms which break down organic matter, and thus more organic soil nitrogen was converted into the soluble form on the limed than on the unlimed series.

If the crops grown on this series had used all of this available nitrogen, then the yields should have been greater on the limed than on the unlimed plots. If the yields were not greater, then more nitrogen must have been lost from the limed than from the unlimed plots, since the former now contain less nitrogen than the latter. That this is so is shown by the averages reported in table 1, a summary of which is given in table 2.

The figures show a distinct loss of nitrogen from both the limed and unlimed sections during the period 1909–1912, but the loss from the limed section is 0.011 per cent more than from the unlimed section. Calculated to the acre basis, this is equivalent to more than 200 pounds of nitrogen for the plowed acre, or taking the first 5 years, an average loss over the unlimed plots of more than 40 pounds a year. There was also a distinct loss of carbon from the limed as compared with the unlimed plots during this period.

The second 5-year period shows a distinct loss in carbon from both series, but a greater loss from the limed than from the unlimed plots. For the nitrogen, on the other hand, a slight gain is shown for this period, but the difference of more than 200 pounds per acre in favor of the unlimed plots still remains.

That this difference in nitrogen content is not made up to the limed plots in a larger return of nitrogen through the crops from these plots is shown by the average yields of nitrogen and dry matter for the 10-year period, as shown in table 3.

TABLE 3

Average pounds of dry matter and nitrogen per acre in crops from limed and unlimed plots

	NITROGEN		DRY MATTER	
	Unlimed	Limed	Unlimed	Limed
	lbs	*lbs*	*lbs*	*lbs*
First 5-year period, 1908–1912*.	40 66	40 76	3657	3669
Second 5-year period, 1913–1917†.	28 31	31 07	2768	2926

* From Bul. 260, N J. Agr. Exp Sta.

† From unpublished results, N. J. Agr. Exp. Sta.

It will be noted that for the first 5-year period, the yields of nitrogen and dry matter are practically the same for the limed and unlimed sections. For the second 5-year period the limed plots show an increase of about 3 pounds of nitrogen and 160 pounds of dry matter per acre over the unlimed plots. However, against this slight increase, there must be set a loss of over 200 pounds of nitrogen per acre for the 10 years, or more than 20 pounds per acre annually. In what way, then, has the lime been a benefit either to the soil or to the crops during the 10-year period? Certainly in this case it has proved a detriment rather than a benefit. The yields on the limed plots have not been appreciably increased and at the end of the 10-year period the soil on these plots contains over 200 pounds per acre less of nitrogen than the soil on the unlimed plots. On the other hand, the acid condition of the unlimed soil acted as a partial preservative of the organic matter, while at the same time there was enough available nitrogen, including that which was applied, to produce a crop about equal to that produced on the limed plots. The limiting factor, whatever it was, affected the crop yields on the two sections about alike, but the reserve supply of nitrogen was being depleted more rapidly on the limed plots.

That the organic matter of the soil must be oxidized or "burned out" with the formation of nitrates and other simple compounds, is inevitable, and it is fortunate that it is so, for in this way available plant-food is formed out of unavailable crop residues and other organic matter, but it is just as inevitable that this loss must be made good in some way if yields are to be increased or even maintained, and the soil kept from wearing out, for the crop residues on light soils are not sufficient to maintain the reserve supply of organic matter.

It would not be safe to say that this will always happen on all types of soil, but it is safe to say that on ordinary well-drained loam soils the tendency will be in this direction if only non-legume crops are grown. It is very evident that on such soils, with modern tillage operations, the oxidation of organic matter is sufficiently rapid without the added stimulus of lime compounds.

There undoubtedly are acid peat soils, and poorly drained heavy clay soils, where there is a large amount of oxidizable organic matter or where oxidation proceeds very slowly, on which lime may be used in connection with the growing of non-legume crops to excellent advantage, but on loams or sandy loams, we may well question the advisability of using lime when non-legumes only are being grown. Under such a system the use of lime adds fuel to the fires of destruction.

The manner of the loss of this nitrogen is still an open question. Certainly a portion of it was lost in the drainage waters, and some escaped into the air as gaseous ammonia or nitrogen, but as to the relative amounts thus lost, we know little. Russell (6), after discussing the various ways by which nitrogen may be lost, says: "The exhaustion of the soil is, therefore, not due to the removal of the crop, but to the cultivation." He then refers to the enormous loss of nitrogen, nearly 70 per cent of the amount applied, from one of the Broadbalk wheat plots which receives 14 tons of stable manure annually. Because of the small amount of drainage water, he thinks the loss of nitrates on account of leaching is small. Russell says further:

> Experiments of this kind have led to the conclusion that some gaseous product is formed in addition to nitrates, and, as no sufficient amount of ammonia can be detected, it is supposed that gaseous nitrogen is given off The conditions for this decomposition appear to be copious aeration, such as is produced by cultivation, and the presence of large quantities of decomposable organic matter. Now these are precisely the conditions of intense farming in old countries and of pioneer farming in new lands, and the result is that the reserves of soil and manurial nitrogen are everywhere being depleted at an appalling rate.

Russell refers to the fact that there are recuperative agencies at work, but adds that the gain in nitrogen does not go on indefinitely. The nitrogen content may be maintained at a high level when the ground is kept in grass and leguminous crops, and at a low level when it is continuously cultivated. "Unfortunately," he concludes, "on our present knowledge, it is impossible to maintain a high content of nitrogen on cultivated land except at a wasteful expenditure of nitrogenous manure."

Nearly forty years ago, Sir John Lawes (1) said very much the same thing but in different words, in speaking of experiments which he and Dr. Gilbert were conducting on Broadbalk field. After discussing the question "what is exhaustion?" he sums up the matter thus:

> We have evidence of a very large loss in the accumulated stock of nitrogen based upon the assumption that my land was originally pasture; there is a further large loss during the period in which the land has been under experiment and growing unmanured corn crops. The crops are declining, the amount of nitrogen removed in the crops each year is declining; and, further, a considerable reduction of the nitrogen in the first 9 inches, with an indication of a small reduction in the second 9 inches, is shown by different analyses of the soil itself.

It may be added that the soil of Broadbalk field is well supplied with carbonate of lime, and this may have contributed to the rapid disappearance of the nitrogen, especially on those parts of the field where legumes were rarely grown.

SUMMARY

Lime in the carbonate form was used on a loam soil at the rate of 1 ton per acre for the first 5 years and 2 tons for the second 5 years in a 5-year rotation of corn, oats, wheat and 2 years of timothy. No legume crops were introduced. Twenty plots with different nitrogen treatment were thus limed and twenty similar plots with parallel nitrogen treatment were left without lime.

The total yields of dry matter and of nitrogen for the 10-year period were essentially the same for the two sections.

Analyses of the soil made soon after the work was started and again at the end of each 5-year period showed that there was a loss of nitrogen from both the limed and unlimed sections. However, the loss from the limed section was distinctly greater than from the unlimed section.

Thus at the end of the 10-year period, there was a positive loss rather than gain from the use of lime.

From this work it would appear that the practice of using lime on light to medium heavy soils, *when leguminous crops are not grown* in the rotation, may be questionable. Under such conditions it is quite possible that a slightly acid reaction may be desirable to prevent the too rapid oxidation of organic matter.

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THE LIME FACTOR IN PERMANENT SOIL IMPROVEMENT

II. ROTATIONS WITH LEGUMES

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In the preceding paper, the authors have discussed the influence of lime on crops grown in a rotation which, so far as possible, excludes all legumes. In this paper, an attempt is made to show the influence of lime in four different rotation systems, all of which include some legume crops.

The soil on which this work was carried out is of the same character as and adjacent to that which was described in the preceding paper. It is a loam inclining to a sandy or gravelly phase and previous to 1908 when the work was started, had been badly neglected.

Twenty-eight $\frac{1}{20}$-acre plots were laid out and these divided into 4 sections of 7 plots each, each section being devoted to a different rotation but all sections having the same lime treatment. The rotations for the different sections are as follows:

	ROTATION 1 PLOTS 21–27	ROTATION 2 PLOTS 28–34	ROTATION 3 PLOTS 35–41	ROTATION 4 PLOTS 42–48
1913	Corn (rye, vetch, crimson clover)	Corn (rye, vetch, crimson clover)	Corn (rye, vetch, crimson clover)	Corn (rye, vetch, crimson clover)
1914	Oats (soybeans and cowpeas)	Potatoes	Potatoes (rye)	Oats and peas, millet
1915	Wheat	Rye	Tomatoes (rye, vetch, crimson clover)	Rye and vetch, rape
1916	Timothy and clover	Timothy and clover	Lima beans (rye, vetch, crimson clover)	Rye, cowpeas (rye)
1917	Timothy and clover	Timothy and clover	Cucumbers (rye and vetch)	Oats and peas, cowpeas

The first plot in each section is the check plot, that is, it receives no lime treatment. The second, third and fourth plots in each section received in 1913, ½, 1 and 2 tons, respectively, of finely ground calcium limestone per acre, and the fifth, sixth and seventh plots received ½, 1 and 2 tons, respectively, of magnesian limestone per acre. This lime treatment was intended to last throughout the 5-year rotation, 1913 to 1917.

Samples of soil were not collected when the work was begun but samples which were taken from three of the plots in 1909 showed an average nitrogen content of a little over 0.10 per cent. In 1913 and again in 1918, samples were collected from all plots for lime-requirement and total nitrogen and carbon determinations. The results of the determinations on the two sets of samples are shown in another place in this paper.

It should be explained here that the results of this work for the first five years, 1908 to 1912, have been published (1, 2), and will not be included in this paper except in so far as they may be needed to make clear the work of the second 5-year period.

In summarizing this work, it was stated that with comparatively few exceptions, both forms of lime increased the yield of dry matter and nitrogen over the yields given by the check plots. It was also pointed out that in the majority of cases, the dry matter of crops from the limed plots showed a higher percentage of nitrogen than dry matter from the unlimed plots.

TABLE 1

Average increase in yield of nitrogen, in pounds per acre, over the check plots, for the non-magnesian and magnesian limestone sections, 1908–1912

		1908	1909	1910	1911	1912
		lbs.	*lbs.*	*lbs.*	*lbs*	*lbs*
Plots 21–27	Non-magnesian limestone	7 95	1 97	5 95	2 00	2 67
(Rotation 1)	Magnesian limestone	14 91	9 70	11.23	10 47	11 70
Plots 28–34	Non-magnesian limestone	10 27	0 00	9 74	2 79	8 55
(Rotation 2)	Magnesian limestone	3 33	0 00	17 17	4 06	5 97
Plots 35–41	Non-magnesian limestone	5 57	3.93	*	8 51	
(Rotation 3)	Magnesian limestone	7.21	5 93	*	6 77	*
Plots 42–48	Non-magnesian limestone	7 71	2 75	52 65	16 74	27 89
(Rotation 4)	Magnesian limestone	6 41	12 73	70 00	0 00	20 05

* Nitrogen determinations not made.

The influence of the lime is clearly brought out by the figures given in table 1 which show the average increase, in pounds of nitrogen per acre, over the check plots, for the two limestone treatments. With only slight exception, a fair gain is shown for all rotations. The most pronounced exception is the potato crop on plots 28 to 34 in 1909.

It is significant that the largest gains are shown for the forage crops rotation, plots 42 to 48. In 1910, on these plots there was a gain of 52.65 pounds of nitrogen per acre for the non-magnesian limestone and 70 pounds per acre for the magnesian limestone. In some cases, the gain is greater for the one form of lime and in other cases for the other form.

The average percentage of nitrogen in the soil samples collected in 1913 is somewhat less than the average of the three samples taken in 1909. This, it is pointed out, appears to indicate a gradual loss of nitrogen even though the plots had received a moderate application of nitrogenous fertilizers and had had the benefit of two or more leguminous crops during the rotation. The land, however, was producing good crops which were with one exception being taken off the land, and thus more nitrogen was being removed in the crops than was being applied in the form of commercial fertilizers.

The second 5-year period embraces the years of 1913 to 1917 and the results are reported for the two forms of lime as yield of dry matter, per cent of nitrogen in the dry matter, and total nitrogen removed in the crop. This plan is carried out for the four rotations and it is thus possible to note the influence of the lime on the yield of dry matter and nitrogen and also to compare the two forms of lime as used in the different quantities.

ROTATION 1—GENERAL FARM CROPS

Plots 21–27—1913 to 1917

This rotation consisted of one year of corn, one of oats, one of wheat and two of timothy and clover. Since this was the beginning of the second 5-year period, there was a timothy and clover sod to be plowed under for corn. Notes made May 1, 1919, state that on the check plot (plot 21, no lime) there was a poor stand of clover in scattered weak bunches and that on the limed plots the stand was fair to good.

The land was fitted for corn and finely ground limestone applied in accordance with the plans already outlined. Fertilizers also were applied on the acre basis as follows:

Acid phosphate, 300 pounds
Muriate of potash, 100 pounds
A mixture of fish and tankage equivalent to 200 pounds of fish

This, it will be noted furnished nitrogen equivalent to about 100 pounds of nitrate of soda.

Reed's Yellow Dent corn was planted on June 2 and this received the usual cultivation during the season. In late September, the corn was harvested, weighed and samples dried and prepared for analysis.

A cover crop of rye, vetch and crimson clover was seeded in the corn at the last cultivation. Notes made in the early spring of 1914 state that the rye on the no-lime plot was about 4 inches high but that there was no vetch or crimson clover on this plot. The rye was thicker on the other plots and there was also more vetch and crimson clover.

On April 14 this cover crop was plowed under and about one week later fertilizers were applied and the plots seeded to oats. The fertilizers were the same as those used for the corn the previous year, except that dried fish was used at the rate of 200 pounds per acre.

In July it was noted that the oats on plot 21 (check) were more mature but the crop was not so heavy as on the lime-treated plots.

On July 27 the oats were harvested and samples prepared for analysis. Following the oats the land was disked and seeded to a mixture of soybeans and cowpeas as a cover crop. The beans and peas grew finely and on September 14 were plowed under preparatory to seeding the wheat. The plants were 6 to 7 inches high and appeared to be well inoculated. Fertilizers were applied as follows:

	lbs. per acre.
Acid phosphate	300
Muriate of potash	100
High-grade tankage	200

On September 30, wheat was seeded at the rate of 7½ pecks per acre. Notes made April 15, 1915, and again on May 1 state that the wheat on plot 21 was not so good as on the other plots. The wheat was harvested July 9, weighed, and samples prepared for analysis. The land was then fitted for timothy and clover and fertilizers applied on the acre basis as follows:

	lbs
Acid phosphate	400
Muriate of potash	100
Nitrate of soda	50

The full application of nitrate was 200 pounds per acre, but 150 pounds of this was reserved for a top-dressing to be applied in the spring. The timothy and clover were seeded on September 2 and this seeding resulted in a good stand. On April 13, 1916, the remainder of the nitrate of soda was applied as a top-dressing. A note made June 1 states that there was practically no clover on plot 21. The crop was harvested July 5, weighed and sampled. On August 22, a second cutting of this crop was made, and it was noted at this time that the timothy was too short to be a factor and that there were only a few bunches of clover on plot 21. The plots that received 2000 pounds of limestone were about three-quarters covered with clover, while those that received 4000 pounds were entirely covered. The crop was weighed and samples prepared for analysis.

On November 9, acid phosphate and muriate of potash were applied to the timothy and clover at the rate of 300 pounds and 100 pounds per acre, respectively.

The following spring nitrate of soda was applied as a top-dressing at the rate of 200 pounds per acre.

On June 1 it was noted that there was not very much clover on any of the plots. This is shown by the low nitrogen content of the hay, analysis of which is reported in table 3. The crop was harvested July 5, weighed and sampled for analysis. As already indicated, samples of all these crops were carefully dried and the yield of dry matter calculated on an acre basis. Nitrogen determinations were made on all samples, thus providing for the calculation of the total nitrogen in the crops.

The yields of dry matter and total nitrogen per acre and the percentage of nitrogen in the dry matter for the various crops for the 5-year period are shown in tables 2, 3 and 4.

Considering first the yields of dry matter as shown in table 2, it will be noted that with only slight exception, the plots receiving the lime treatment have given better yields than the check plots. The most notable exception is the oats crop of 1914, where the check plot yielded more grain than any of the treated plots. In the case of the other crops the check plot invariably yielded less than the treated plots.

TABLE 2

Rotation 1—Yield of dry matter, in pounds per acre, for 5 years, 1913-1917

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914		1915		1916		1917	TOTAL		ANNUAL INCREASE OVER CHECK	
		Corn		Oats		Wheat		Timothy and clover	Red clover	Timothy and clover	Grain	Stover straw hay	Grain	Stover straw hay
		Grain	Stover	Grain	Straw	Grain	Straw							
	lbs	*lbs*	*lbs*	*lbs*	*lbs*	*lbs.*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs.*
21	Check	850	2650	660	860	1040	1240	3600	200	2620	2550	11,170		
Calcium-limestone section														
22	1000	1225	2800	480	800	1200	1440	3680	980	3520	2905	13,220	118.3	110
23	2000	1400	2950	600	1040	1260	1640	4380	1420	3640	3260	15,070	236.7	780
24	4000	1675	3150	600	1020	1240	1480	4400	1660	3500	3515	1 ,210	321.7	808
Average		1433	2967	560	953	1233	1520	4153	1353	3553	3227	14,500	225.6	666
Magnesian-limestone section														
25	1000	1850	3500	620	1040	1240	1600	4440	1100	3520	3210	15,200	220.0	806
26	2000	1875	3500	600	1120	1260	1680	4620	1920	3800	3735	16,640	395.0	1094
27	4000	2000	3200	640	1040	1220	1600	4540	1800	4220	3860	16,400	436.7	1046
Average		1742	3400	620	1067	1240	1627	4533	1607	3847	3602	16,080	350.6	982

With very slight exception, there is some increase in yield with each increase in the amount of calcium limestone applied, and likewise an increase from the 1000 to the 2000-pound application of magnesian limestone. On the other hand, there is not so often an increase in yield from the 2000 to the 4000-pound application in this section. It is to be noted, however, that the average yields on the magnesian-limestone section are without exception, larger than the corresponding yields for the calcium-limestone section.

The average *annual* increase for the check plots for the calcium-limestone section is for the grain 225.6 pounds, and for the straw, hay, etc., 666 pounds, whereas the corresponding figures for the magnesian-limestone section are

TABLE 3

Rotation 1—Yield of nitrogen, in pounds per acre, removed by crops for the years 1913–1917

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914		1915		1916		1917	TOTAL NITROGEN GRAIN, HAY, ETC.	ANNUAL INCREASE OF NITROGEN OVER CHECK
		Corn		Oats		Wheat						
		Grain	Stover	Grain	Straw	Grain	Straw	Timothy and clover	Red clover	Timothy and clover		
	lbs.	*lbs*	*lbs.*	*lbs.*	*lbs*	*lbs.*	*lbs*	*lbs*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
21	Check	10.31	15 30	13 36	6 29	20 47	3 66	31 18	4 32	19 91	124 80	
	Calcium-limestone section											
22	1000	15 35	17 76	10 48	6 56	24 30	3 96	41 29	25 03	28 51	173 24	9 69
23	2000	18 37	21 90	13 27	8 90	25 16	6 30	52 74	35 87	30 90	213 41	17 72
24	4000	24 12	25 21	13 16	7 64	24 63	4 07	52 58	44 97	29 72	226.10	20 26
Average		19 28	21 62	12 30	7 70	24 70	4 78	48 87	35 29	29 71	204 25	15 89
	Magnesian-limestone section											
25	1000	17 17	23 99	13 60	7 88	25 13	5 34	45 78	28 90	27 81	195 60	14 16
26	2000	25 71	26 57	13 10	10 28	24 80	4 79	54 79	51 30	32 26	243 60	23 76
27	4000	29 78	24 40	15 12	8 90	24 24	5 50	52 16	47 61	34 56	242 27	23 49
Average		24 22	24 99	13 94	9 02	24.72	5.21	50 91	42 60	31 54	227 16	20 47

TABLE 4

Rotation 1—Per cent of nitrogen in dry matter of crops for the years 1913–1917

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914		1915		1916		1917
		Corn		Oats		Wheat		Timothy and clover	Red clover	Timothy and clover
		Grain	Stover	Grain	Straw	Grain	Straw			
	lbs	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
21	Check	1 213	0 573	2 024	0 731	1 968	0 295	0 866	2 161	0 760
	Calcium-limestone section									
22	1000	1 253	0 652	2 184	0 820	2 025	0 275	1 122	2 554	0 810
23	2000	1 312	0 761	2 211	0 856	1 997	0 384	1 204	2 526	0 849
24	4000	1 440	0 820	2 193	0 749	1 987	0 275	1 195	2 709	0 849
Average		1 335	0 744	2 196	0 808	2 003	0 311	1 174	2 596	0 836
	Magnesian-limestone section									
25	1000	1 272	0 711	2 193	0 758	2 027	0 334	1 031	2 627	0 790
26	2000	1 371	0 790	2 184	0 918	1 968	0 285	1 186	2 672	0.849
27	4000	1 489	0 810	2 362	0 856	1 987	0 344	1 149	2 645	0 819
Average		1 377	0 770	2 246	0 844	1 994	0 321	1 122	2 648	0 819

for the grain 350.6 pounds, and straw, hay, etc., 982 pounds. Attention should be called to the very wide difference between the yield of red clover on the check plot and the treated plots for 1916: This crop, as is well known, is especially susceptible to the unfavorable soil condition brought about by the deficiency of lime.

What has been said with reference to the yield of dry matter will apply in most cases to the yield of total nitrogen shown in table 3. With very few exceptions, the lime treatments have resulted in an increase of total nitrogen over the check plot. The 2000-pound application of both forms of lime, in nearly all cases, shows an increase over the 1000-pound application. In a number of cases, on the other hand, the 4000-pound application shows no increase over the 2000-pound application. The total yields of nitrogen for the 5 years are as follows:

	LIMESTONE APPLIED	YIELD
	lbs per acre	*lbs per acre*
Check		124.8
Calcium limestone	1000	173.2
	2000	213.4
	4000	226.1
Magnesian limestone	1000	195.6
	2000	243.5
	4000	242.3

From these figures it will be noted that with the calcium limestone 4000 pounds gave a slightly higher yield than 2000 pounds. With the magnesian limestone, the yields with the 2000 and 4000-pound applications are practically the same.

The annual increase for the calcium limestone plots over the check plots ranges from 9.69 pounds, with the 1000-pound application to 20.26 pounds with the 4000-pound application. In the case of the magnesian limestone the increase is 14.16 pounds with the 1000-pound application, 23.76 pounds with the 2000-pound application, and 23.49 pounds with the 4000-pound application. Excluding the 1000-pound applications, the increases per acre will average above 20 pounds per year. Thus the increase for one year would more than pay the cost of the limestone.

The percentage of nitrogen in the dry matter as reported in table 4 is of especial interest. With very few exceptions, it is higher in the samples from the limed plots than in those from the check plots. This is true of the grain and also of the straw, corn stover and hay. In earlier publications we have called attention to this effect of lime and have suggested, in the case of the legume crops, that the lime tends to establish soil conditions favorable to the development of those organisms which aid the plant in storing up atmospheric nitrogen. The higher percentage of nitrogen in non-leguminous plants is no doubt in part an indirect effect of the stimulation of the symbiotic organisms, that is, the utilization of nitrogen which was secured through leguminous plants, and in part the result of the stimulation of those organisms which nitrify organic matter in general.

To increase the crop yield means much, but to increase also the nitrogen content of the crop adds still further to its total value.

The average percentages of nitrogen in the crops from the two sections differ but slightly, that is, the average percentage of nitrogen in the crops from the magnesian-limestone section is not appreciably greater than the average in crops from the calcium-limestone section.

Referring again to the total yield of nitrogen (table 3) it may be pointed out that with 2000 pounds per acre of magnesian limestone, the timothy and clover crop of 1916 removed slightly more than 100 pounds of nitrogen per acre. In comparison with this, it will be noted that the corn crop of 1913—grain and stover—removed about one-half as much nitrogen. The smallest amount of nitrogen was removed by the oat crop of 1914. This was somewhat below the normal, because the oats shattered badly at the time of harvesting.

The fertilizers which were applied during the 5 years carried a total of about 108 pounds of nitrogen. The crops on the calcium-limestone section removed an average during the same period of a little more than 200 pounds per acre, and those on the magnesian-limestone section removed an average of nearly 230 pounds per acre. There is good reason for believing that this excess over the amount applied is being drawn from the atmosphere largely through the legume crops. If it is not being thus secured then the soil is gradually being depleted of its nitrogen supply. This question will be further discussed in connection with the table showing the percentage of nitrogen in the soil.

ROTATION 2—GENERAL FARM CROPS WITH POTATOES

Plots 28–34—1913 to 1917

This rotation consists of one year of corn, one of potatoes, one of wheat and two of timothy and clover. As in the case of rotation 1, plots 21 to 27, the timothy and clover sod preceded the corn and the corn crop was fertilized and cared for in the same way as in rotation 1. Early in August a cover crop of rye, vetch and crimson clover was seeded in the corn. Notes taken April 8, 1914, show that the rye was thin on plots 28 and 29, and also that there was not much vetch and crimson clover on these plots. This cover crop was plowed under about the middle of April, 1914, and fertilizers applied on the acre basis as follows:

	lbs.
Acid phosphate	300
Muriate of potash	100
Dried fish	200

Early in May the plots were planted to Green Mountain potatoes. After these had been planted, it was decided that the first application of fertilizers was not sufficient and the following additional application was made per acre—100 pounds of acid phosphate and 200 pounds of muriate of potash, thus making the total application 400 pounds of acid phosphate, 300 pounds of muriate of potash and 200 pounds of dried ground fish. This fertilizer was spread broadcast.

The crop was cultivated and sprayed in the usual way throughout the season. Potatoes were dug about the twenty-fifth of August and later the plots were disked preparatory to seeding to rye. Fertilizers were applied on the acre basis as follows:

	lbs.
Acid phosphate	300
Muriate of potash	100
High-grade tankage	200

The plots were seeded to rye at the rate of 6 pecks per acre (vetch and crimson clover were not included in this seeding).

Notes taken April 15, 1915, state that the rye was 8 to 10 inches high at that time. The rye was harvested as grain the last of June and about the last of August the land was prepared and seeded to timothy and clover. For this crop fertilizers were applied on the acre basis as follows:

	lbs.
Acid phosphate	400
Muriate of potash	100
Nitrate of soda	50

On April 13, 1916, these plots received a further top-dressing of nitrate of soda equivalent to 150 pounds per acre. On June 6, the timothy on these plots was about 22 to 24 inches high. There was very little clover on the check plot (no. 28), though there was a fine stand on the other plots. On July 5 the crop was harvested and weighed and the samples prepared.

On August 1 the second growth of clover on these plots was reported as about 12 to 15 inches high, but it was noted that there was very little on plot 28. This second growth was cut and weighed and samples prepared August 23. On November 9 the plots were fertilized at the following rate per acre:

	lbs.
Acid phosphate	300
Muriate of potash	100

On April 12, 1917, nitrate of soda was applied at the rate of 200 pounds per acre, no nitrate having been applied the previous fall. The effect of this top-dressing was soon apparent, and the timothy made a good growth. The clover was poor. The crop was harvested and weighed July 5 and samples prepared for analysis. The yield of dry matter and total nitrogen from these plots and the percentage of nitrogen in the dry matter are reported in tables 5, 6 and 7.

The yields of dry matter for the 5-year period are summarized in table 5. For the corn and the timothy and clover the lime treatment shows fair increases over the check plots. For the potatoes and rye—1914 and 1915—the yields on the treated plots are slightly less than those on the check, with the exception of the potatoes on the calcium limestone section. The failure of these crops to respond to the lime treatment was probably due to the partial failure of the cover crop which preceded the potatoes, and the absence of any legume cover crop preceding the rye. Or to express the matter another way, the lime could not exert its full benefit in the partial or complete absence of those crops through which it manifests itself most readily. The most notable increases occur with the mixed timothy and clover and clover alone. The latter shows an increase of more than 100 per cent with the magnesian limestone and almost as much with the calcium limestone.

TABLE 5

Rotation 2—Yield of dry matter, in pounds per acre, for the 5-year period, 1913–1917*

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914	1915		1916		1917	TOTAL†		YEARLY INCREASE OVER CHECK	
		Corn			Rye								
		Grain	Stover	Potatoes	Grain	Straw	Timothy and clover	Red clover	Timothy and clover	Grain	Forage, hay, etc	Grain	Stover, hay
	lbs	lbs	lbs.	lbs	lbs	lbs	lbs	lbs.	lbs.	lbs	lbs.	lbs	lbs
28	Check	1050	2425	6200	1400	2680	3920	660	3500	2450	13,185		
Calcium-limestone section													
29	1000	1850	2575	7040	1360	2600	4320	960	3680	3210	14,135	380	238
30	2000	1900	2425	7100	1400	2700	4820	1160	4440	3300	15,545	425	590
31	4000	2025	2725	6560	1280	2560	4520	1580	4500	3305	15,885	428	675
Average		1925	2575	6900	1347	2620	4553	1233	4207	3272	15,188	411	501
Magnesian-limestone section													
32	1000	2000	2700	5960	1440	2600	4620	1080	3960	3440	14,960	495	444
33	2000	2050	2700	5620	1520	2800	4440	1760	3840	3570	15,540	560	589
34	4000	2200	2525	5240	1320	2560	4200	1660	3580	3520	14,525	535	335
Average.		2083	2642	5607	1427	2653	4420	1500	3793	3510	15,008	530	456

* Field weight of potatoes.

† Potatoes not included in total.

The yields of total nitrogen which are shown in table 6 follow quite closely the yields of dry matter. In the case of the corn (grain) and the red clover the increase amounts to over 100 per cent. The potatoes and rye on the check plot yielded very nearly the same amount of nitrogen as on the treated plots.

On the calcium-limestone plots, there was recovered an average of nearly 228 pounds of nitrogen per acre for the 5-year period and on the magnesian-limestone plots an average of about 234 pounds for the same period, or more than 45 pounds per acre per annum. During the 5 years, there was applied to these plots about 108 pounds of nitrogen, or 22 pounds per year. Apparently we have here a loss of about 24 pounds per acre annually, but undoubtedly this loss was more than made up by the nitrogen secured through the legume crops.

Table 7 shows the percentage of nitrogen in the dry matter of the different crops. It will be noted that, with very few exceptions, the crops from the lime-treated plots—both sections—show a higher percentage of nitrogen than the crops from the check plots. This is in accord with the results secured in rotation 1.

ROTATION 3—CORN, POTATOES AND MARKET GARDEN CROPS

Plots 35-41—1913 to 1917

The corn in this rotation was preceded by the timothy and clover sod and received the same treatment as that in rotations 1 and 2, and the potatoes the same treatment as those in rotation 2. Following the potatoes, the plots were disked, fertilizers applied and rye seeded at the rate of 6 pecks per acre. The fertilizer treatment was the same as that given to plots 28 to 34 at this time.

On April 15, 1915, the rye on these plots was about 8 to 10 inches high. On May 3, the plots were disked preparatory to plowing under the rye as a green manure. The plots were plowed on June 2 and fertilizers applied for the tomatoes on the acre basis as follows:

	lbs.
Acid phosphate	600
Muriate of potash	300
Nitrate of soda	200
Dried blood	160

Chalk's Early Jewel tomatoes were set out and later in the season received the usual cultivation and other attention. Picking began about the middle of August and continued until the middle of September, five pickings being made.

Unfortunately, samples were not saved from each plot but a composite sample was made. Thus it is necessary in calculating the nitrogen removed by the tomatoes to base the calculations on the analysis of this composite sample.

Following the tomatoes, the land was disked and seeded to a mixture of rye, vetch and crimson clover. The amount of rye in the mixture was small and consequently a thin stand was secured. Notes made on April 1, 1916,

TABLE 6

Rotation 2—Yield of nitrogen, in pounds per acre, removed by crops for the years 1913–1917

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914	1915		1916		1917	TOTAL NITROGEN	YEARLY INCREASE OVER CHECK
		Corn		Potatoes	Rye		Timothy and clover	Red clover	Timothy and clover		
		Grain	Stover		Grain	Straw					
	lbs	*lbs*	*lbs*	*lbs.*	*lbs*	*lbs,*	*lbs*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
28	Check	14 29	19 00	22 47	19 96	8 23	38 96	14 93	27 30	165 14	
	Calcium-limestone section										
29	1000	28.29	22 31	23 84	19 53	7 20	38 62	23 03	29 44	192 26	5 42
30	2000	32 98	28.17	20 90	21 21	8 29	56 25	26 45	39 03	233 28	13 63
31	4000	35 37	35 06	20 34	20 15	8 60	62 24	39 63	36 00	257.39	18.45
Average		32 21	28 51	21.69	20 30	8 03	52 37	29 70	34 82	227 64	12 50
	Magnesian-limestone section										
32	1000	31 36	25 16	21 72	21 40	7.98	52 67	26 49	34 41	221 19	11 21
33	2000	31 92	30 20	21 14	21 98	8.60	46 18	44 62	37 52	242 16	15 40
34	4000	34 06	26 79	19 26	19 87	8.60	55.15	41 48	34 65	239 86	14 94
Average . .		32 45	27 38	20 71	21 08	8 39	51 33	37 53	35 53	234 40	13 85

TABLE 7

Rotation 2—Per cent of nitrogen in dry matter of crops for the 5-year period, 1913–1917

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914	1915		1916		1917
		Corn		Potatoes	Rye		Timothy and clover	Red clover	Timothy and clover
		Grain	Stover		Grain	Straw			
	lbs	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
28	Check	1 361	0 751	1 783	1 426	0 307	0 994	2 262	0 780
	Calcium-limestone section								
29	1000	1 529	0 830	1 703	1 436	0 277	0 894	2 399	0 800
30	2000	1 736	1 097	1 560	1 515	0 307	1 167	2 280	0 879
31	4000	1 746	1 235	1 614	1 574	0 336	1 377	2.508	0 800
Average. .		1 670	1 054	1 626	1.508	0 307	1 146	2 396	0 826
	Magnesian-limestone section								
32	1000	1 568	0 889	1 872	1 486	0 307	1 140	2 453	0.869
33	2000	1 557	1 067	1 854	1 446	0 307	1 040	2 535	0 977
34	4000	1 548	0.998	2 006	1 505	0 336	1 313	2 499	0.968
Average. .		1 558	0.985	1 911	1 479	0 317	1 164	2 496	0 938

state that the cover crop on these plots was fair. Later notes show that the vetch and crimson clover were poor on plot 35 and very good on plot 41.

On May 8 this cover crop was plowed under and on the nineteenth fertilizers were applied on the acre basis as follows:

	lbs
Acid phosphate	600
Muriate of potash	300
Nitrate of soda	100

About one week later, Burpee's bush lima beans were planted. Notes made July 1 show that the beans were somewhat irregular in growth. On August 1 they were in bloom. On the tenth of August the cover crop consisting of rye, vetch and crimson clover was seeded in the beans. Between September 11 and October 20, four pickings of beans were made. Notes made April 12, 1917, show that there was a good cover crop on these plots. On May 15 the cover crop was plowed under and fertilizers applied on the acre basis as follows:

	lbs
Acid phosphate	400
Muriate of potash	100
Nitrate of soda	160
Tankage	200

On the twenty-fifth of May the plots were planted to White Spine cucumbers. These received the usual cultivation and other attention during the summer. A number of pickings were made between July 27 and August 31. Samples were taken from each plot for nitrogen determinations.

After the cucumbers were off the plots were disked and seeded to rye and vetch.

The yields of total dry matter (or marketable vegetables), the total nitrogen and percentage of nitrogen in the dry matter (or marketable vegetables) are shown in tables 8, 9 and 10.

From the figures given in table 8 it will be noted that the limestone-treated plots, in nearly all cases, show a distinct increase over the check plots. This is true for both forms of limestone and if we may judge from the averages, one form does not have much advantage over the other. The cucumbers on the limed plots show notable increases over the check plots. The yield of potatoes on the calcium-limestone section is greater than the yield on the magnesian-limestone section. This is true also of the potatoes grown in rotation 2. It is doubtful, however, if the differences are sufficient to have any particular significance.

It should be noted that the yields of potatoes, tomatoes and cucumbers are the yields at the time of harvesting and not the yields of dry matter.

Table 9 shows the total yield of nitrogen for the various crops. With only one exception (lima beans on plot 41) the lime-treated plots show pronounced increases over the check plots.

TABLE 8

Rotation 3—Yield of dry matter (or harvesting weights), in pounds per acre, for the years 1913–1917

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914	1915	1916		1917	TOTAL		
		Corn		Potatoes	Ripe tomatoes	Lima beans		Marketable cucumbers	Grain	Stover	Potatoes tomatoes cucumbers
		Grain	Stover			Seeds	Pods				
	lbs.	*lbs*	*lbs*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
35	Check	1475	2650	4800	24,080	480	260	8,138	1955	2910	37,018
					Calcium-limestone section						
36	1000	1675	2850	6320	28,550	660	332	11,618	2335	3182	46,488
37	2000	1750	3125	6300	27,860	860	448	13,536	2610	3573	47,696
38	4000	2025	3325	5740	25,610	720	360	11,688	2745	3685	43,038
Average. .		1817	3100	6120	27,340	747	380	12,281	2563	3480	45,741
					Magnesian-limestone section						
39	1000	1775	2975	5680	25,454	540	300	13,432	2315	3275	44,566
40	2000	2100	3275	6260	27,720	660	288	13,482	2760	3563	47,462
41	4000	2200	3200	5100	28,924	360	200	13,496	2560	3400	47,520
Average.		2025	3150	5680	27,366	520	263	13,470	2545	3413	46,516

NOTE: Potatoes, tomatoes and cucumbers weighed as harvested.

TABLE 9

Rotation 3—Yield of nitrogen, in pounds per acre, removed by crops for the years 1913–1917

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914	1915	1916		1914	TOTAL NITROGEN	YEARLY INCREASE OVER CHECK
		Corn		Potatoes	Ripe tomatoes	Lima beans		Marketable cucumbers		
		Grain	Stover			Seeds	Pods			
	lbs.	*lbs*	*lbs*	*lbs*	*lbs*	*lbs.*	*lbs*	*lbs.*	*lbs.*	*lbs.*
35	Check	22 11	20 87	15 42	40 55	14 52	2 17	9 50	125 14	
					Calcium-limestone section					
36	1000	25 61	23 50	16 20	48 08	19 97	2 46	14 82	150 64	5 1
37	2000	27 27	26 12	19 14	46 92	26 33	3 03	18 34	167 15	8 4
38	4000	30 76	24 62	20 74	43 13	20 51	2 30	14.96	157 02	6 4
Average		27 88	24 75	18 69	46 04	22 27	2 60	16 04	158 27	6 6
					Magnesian-limestone section					
39	1000	24 51	22 01	16 41	42 86	16 74	2 56	16 94	142 03	3 4
40	2000	30 87	25 41	19 25	46 68	20 76	2 40	16 94	162 31	7.4
41	4000	34 06	27.99	16 34	48 71	11 43	2 88	15 86	157 27	6 4
Average.		29 81	25 14	17 33	46 08	16 31	2 61	16.58	153 87	5.7

The averages for the two sections run very close together, though the average of the potatoes for the calcium-limestone section has a slight advantage over the corresponding average for the magnesian-limestone section.

Attention should be called to the fact that the total amount of nitrogen recovered in the crop is less, by more than fifty pounds, than the total amounts recovered from the two preceding rotations. This calls attention to the fact that market garden crops, although generally giving larger and more profitable yields than general farm crops, take from the land less nitrogen than the latter.

TABLE 10

Rotation 3—Per cent of nitrogen in dry matter of crops for the 5-year period, 1913–1917

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914	1915	1916		1917
		Corn		Potatoes*	Ripe tomatoes	Lima beans		Marketable cucumbers†
		Grain	Stover			Seeds	Pods	
	lbs.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
35	Check	1 499	0 820	1 676	0 1684‡	3 025	0 835	0 121
Calcium-limestone section								
36	1000	1 529	0 860	1 373	0 1684	3 025	0 742	0 133
37	2000	1 558	0 879	1 569	0 1684	3 062	0 677	0 141
38	4000	1 519	0 769	1 819	0 1684	2 849	0 640	0 130
Average		1 535	0 836	1 587	0 1684	2 979	0 686	0 135
Magnesian-limestone section								
39	1000	1 381	0 771	1 578	0 1684	3 100	0 854	0 135
40	2000	1 470	0 810	1 631	0 1684	3 146	0 835	0 128
41	4000	1 548	0 939	1 738	0 1684	3 174	1 438	0 124
Average		1 466	0 840	1 649	0 1684	3 140	1 042	0 129

* Potatoes dried.

† Tomatoes and cucumbers sampled as harvested.

‡ Composite sample.

The total amount of nitrogen applied to these crops during the 5 years was about 150 pounds, or 30 pounds per acre, while the average amount removed on the lime-treated sections was about 155 pounds, or a yearly average of about 31 pounds per acre.

Table 10 shows the percentage of nitrogen in the different crops. The increase in percentage due to the lime treatment is not so consistent as in rotations 1 and 2, but there are as many plots for which the percentage of nitrogen is above that of the check as there are plots with the percentage below the check. The nitrogen determinations were made on the potatoes after they had been thoroughly dried but, as already explained, they were made on the tomatoes and cucumbers as harvested.

ROTATION 4—FORAGE CROPS

Plots 42–48—1913 to 1917

The corn which was planted on these plots in 1913 had been preceded in 1912 by oats and peas followed by cowpeas, both of which crops were taken off. As no winter cover crop was seeded, the plots remained bare during the winter of 1912 and 1913. This corn received the same attention and treatment as the corn in the other rotations. On August 6 a cover crop of rye, vetch and crimson clover was seeded in the corn. On April 21, 1914, this cover crop was plowed under, and notes made at that time state that there was a fair crop of vetch and crimson clover on all plots except plot 42 which had none. On April 23, fertilizers were applied to the oats and peas at the following rate:

	lbs. per acre
Acid phosphate	300
Muriate of potash	100
Fish	200

On April 24 oats and peas were seeded at the rate of 3 bushels per acre. On July 6 these were cut as a forage crop and the land immediately prepared for seeding to barnyard millet. The millet was seeded July 8. Notes made under date of August 4 state that there was a thin stand and that weeds were coming in to the detriment of the millet. The millet was cut August 28, the weights recorded and samples prepared for analysis. On August 31, the plots were plowed and prepared for winter vetch. Fertilizers were applied at the following rate per acre:

	lbs.
Acid phosphate	300
Muriate of potash	100

Notes made April 15, 1915, state that there was a very poor stand of vetch on plot 42. The vetch was good on the other plots. The poor stand on plot 42 was no doubt the result of winter-killing due to adverse soil conditions. On June 1, it was estimated that there was only one-fourth of a stand on this plot. On the other plots, the vetch was 20 to 26 inches high. The vetch was cut June 9 and the ground plowed and seeded to Dwarf Essex rape. Fertilizers were applied on the acre basis as follows:

	lbs.
Acid phosphate	300
Muriate of potash	100
Nitrate of soda	200

The rape was harvested September 15 but was not an entirely satisfactory crop on account of a rather heavy growth of crab-grass. The land was again plowed and seeded to rye, fertilizers being applied on the acre basis as follows:

	lbs.
Acid phosphate	400
Muriate of potash	100
Nitrate of soda	50

On November 1 a good stand of rye was noted on all the plots.

On April 13, 1916, a top-dressing of nitrate of soda was made at the rate of 150 pounds per acre. The rye made a good growth and on June 1 was cut as a forage crop. The land was plowed, fertilized and seeded to Whippoorwill cowpeas. The fertilizer treatment consisted of acid phosphate at the rate of 400 pounds and muriate of potash at the rate of 200 pounds per acre. Notes made August 1 state that there was a thin stand of the cowpeas and that there was much crab-grass among them. On September 19 the cowpeas were cut for hay The record shows that the crop was about 50 per cent crab-grass. On September 28 the plots were disked and seeded to rye at the rate of 4 pounds per plot. A good stand was secured On April 17, 1917, the rye was plowed under and fertilizers applied at the following rate per acre:

	lbs.
Acid phosphate	300
Muriate of potash	100
Dried fish	200

Oats and peas were seeded at the rate of 3 bushels per acre On July 1 the oats and peas were about 8 to 10 inches high but the check plot was noted as being the poorest They were cut as hay July 12 and the ground disked and seeded to Whippoorwill cowpeas at the rate of 6 pounds per plot Notes of August 1 state that there was a good stand The check plot, however, was the poorest. On October 2 they were harvested as hay The plots were prepared with a spring-tooth harrow on October 12 and rye was seeded at the rate of 4 pounds per plot The yields for these plots during the 5-year period, together with the total yield of nitrogen and percentage of nitrogen in the crop are shown in tables 11, 12 and 13

The yield of dry matter for the 5-year period is shown in table 11. Again, with slight exception, the lime-treated plots show a distinct increase over the check plots. In the calcium-limestone section, the oats and peas grown in 1914 show an average increase of 760 pounds per acre and the vetch grown the next year shows a much larger average increase It may be explained here that the vetch on the check plot was almost an entire failure

In most cases the averages for the magnesian limestone section run quite close to those for the calcium-limestone section. However, for the millet the yield on the former is less than on the latter. For the vetch of 1915 the yield is almost twice as much from the magnesian-limestone section as from the calcium-limestone section. The average yearly increase in grain for the calcium-limestone section amounts to 533 pounds and for the magnesian-limestone section to 258 pounds. The corresponding figures for the corn stalks and hay are 1021 and 1073 pounds. This is equivalent to 4⅝ to 9½ bushels of grain and ½ ton of stalks and hay per acre, which represents the annual gain over the no-lime plot.

The yield of nitrogen for this rotation is shown in table 12. In nearly all cases the lime treatment has resulted in a substantial increase in yield of nitrogen over the check plot. The most pronounced exception is the millet in the magnesian-limestone section in 1914. It should be recalled that this crop came near being choked out by crab-grass, and this accounts in part for the low yields. Attention may be called to the high yield of nitrogen from the vetch crop of 1915. For the calcium-limestone section, there is an average of 48 pounds per acre, and for the magnesian-limestone section an average of nearly 79 pounds per acre.

TABLE 11

Rotation 4—Yield of dry matter, in pounds per acre, for the 5-year period 1913–1917

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914		1915		1916		1917		TOTAL		YEARLY INCREASE OVER CHECK	
		Corn													
		Grain	Stover	Oats and peas	Millet	Vetch	Rape	Rye	Cowpeas	Oats and peas	Cowpeas	Grain	Forage, hay, etc	Grain	Stover and hay
	lbs	*lbs*	*lbs*	*lbs.*	*lbs.*	*lbs*	*lbs*	*lbs.*	*lbs*	*lbs*	*lbs.*	*lbs.*	*lks.*	*lbs*	*lbs*
42	Check	1350	2675	1520	400	220	1700	2240	1940	2120	1160	1350	13,975		
Calcium-limestone section															
43	1000	1775	2825	2200	480	840	1840	2660	2240	2600	1240	1775	16,925	425	590
44	2000	1825	3000	2200	620	1400	2120	2780	2540	2820	1600	1825	19,080	475	1021
45	4000	2050	3275	2440	520	2160	2120	3120	2880	2680	2040	2050	21,235	700	1452
Average .		1883	3033	2280	540	1467	2027	2853	2553	2700	1627	1883	19,080	533	1021
Magnesian-limestone section															
46	1000	1550	2425	2120	140	2180	1660	2880	2360	3040	1440	1550	18,245	200	854
47	2000	1625	2900	2080	280	2920	2040	2960	2780	2940	1480	1625	20,380	275	1281
48	4000	1650	2550	1920	300	2960	2460	2400	2380	2660	1760	1650	19,390	300	1083
Average ..		1608	2625	2040	240	2687	2053	2747	2507	2880	1560	1608	19,338	258	1073

The highest yield—nearly 90 pounds—was on the magnesian-limestone plot which received 4000 pounds per acre. Next to the vetch, the oats and peas and the cowpeas gave the largest yields of nitrogen. In 1917, the total nitrogen secured for these two crops on the magnesian-limestone section amounts to more than 100 pounds for each limestone treatment.

The total nitrogen for the 5 years averages 373⅔ pounds for the calcium-limestone section and 381⅓ pounds for the magnesian-limestone section, or more than 75 pounds per year. There was applied to these plots a total of about 108 pounds of nitrogen per acre for the 5 years, or nearly 22 pounds per year.

TABLE 12

Rotation 4—Pounds of nitrogen per acre removed by crops for the 5-year period 1913–1917

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914		1915		1916		1917		TOTAL NITROGEN IN GRAIN, HAY, ETC.	YEARLY INCREASE IN NITROGEN OVER CHECK
		Corn		Oats and peas	Millet	Vetch	Rape	Rye	Cow-peas	Oats and peas	Cow-peas		
		Grain	Stover										
	lbs	*lbs*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs*
42	Check	18 37	18 58	25 20	4 74	7 08	16 68	23 50	28 52	27 41	32 31	202 39	
	Calcium-limestone section												
43	1000	25 03	20 29	41 18	6 33	28 67	18 42	29 47	28 34	42 61	34 77	275 11	14.5
44	2000	26 65	25 46	41 58	8 79	49 62	22 09	28 36	41 12	49 01	46.72	339 40	27 4
45	4000	28 52	26 91	46 80	7 28	65 82	24 38	36 07	59 73	56 09	64 87	416 47	42 8
Average.. .		26 73	24 22	43 18	7 47	48 04	21 63	31 30	43 06	49.24	48 79	373 69	28.23
	Magnesian-limestone section												
46	1000	21 56	18 19	37 91	2 22	60 08	19 24	30 79	54 89	57 64	47 19	349 71	29 5
47	2000	22 44	20 58	35 65	3 79	86 52	23 26	30 49	65 44	54 57	50 35	393 09	38 1
48	4000	24 26	20 52	38 78	4 07	89.63	27 08	23 09	54 91	64 08	55 30	401 72	39 9
Average...		22.75	19 76	37 45	3 36	78 74	23 19	28.12	58 41	58 76	50 95	381 51	35 83

TABLE 13

Rotation 4—Per cent of nitrogen in dry matter of crops for the year. '913–1917

PLOT	LIMESTONE APPLIED PER ACRE	1913		1914		1915		1916		1917	
		Corn		Oats and peas	Millet	Vetch	Rape	Rye	Cow-peas	Oats and peas	Cow-peas
		Grain	Stover								
	lbs.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
42	Check	1 361	0 721	1 658	1.186	3 216	0 981	1 049	1 470	1 203	2 785
	Calcium-limestone section										
43	1000	1 410	0 751	1 872	1 319	3.413	1 001	1 108	1 265	1 639	2 804
44	2000	1 460	0 899	1.890	1 417	3 544	1 042	1 020	1 619	1 738	2 920
45	4000	1 391	0 860	1 918	1 400	3 047	1 150	1 156	2 074	2 093	3.180
Average ..		1 420	0 837	1 893	1 379	3 335	1 064	1 095	1 653	1 823	.2 968
	Magnesian-limestone section										
46	1000	1 391	0 790	1 788	1 587	2 756	1 159	1 069	2 326	1 896	3.277
47	2000	1 381	0 731	1 714	1 355	2 963	1 140	1 030	2 354	1 856	3.402
48	4000	1 470	0 860	2.020	1 355	3 028	1 101	0 962	2 307	2.409	3.142
Average		1 414	0.794	1 841	1 432	2.916	1 133	1 020	2.329	2 054	3 274

Thus there was removed in the crop more than three times as much as was applied in the fertilizers. The average annual increase over the check plot for the calcium-limestone section is 28 pounds per acre and for the magnesian-limestone section 35 pounds per acre. These increases are equal to, and in some cases exceed, the total yields in some of the wheat and rye crops reported in rotation 1.

The percentage of nitrogen in the dry matter is shown in table 13. In the majority of cases there is a higher percentage in samples from the lime-treated plots than in those from the check plots. This is well brought out by comparing the percentage as shown for the check plots with the corresponding averages for the two lime-treated sections. With only two exceptions the latter show a higher percentage of nitrogen in the dry matter than does the check plot. The high percentage of nitrogen in the vetch of 1915 and the cowpeas grown in 1917 is noteworthy, the highest analysis with vetch being 3.54 per cent and the highest with cowpeas 3.40 per cent. The oats and peas show an average close to 2 per cent, while the non-legume crops such as corn, rape and rye show an average of a little over 1 per cent for the rye and rape and 1.4 per cent for the corn and millet.

These figures present a strong argument for growing leguminous crops wherever they can be brought into the rotation to good advantage.

THE INFLUENCE OF THE LIME TREATMENT ON THE NITROGEN CONTENT AND LIME REQUIREMENT OF THE SOIL

Lime requirement

In connection with the foregoing discussion, the figures presented in table 14 are of interest. At the end of each 5-year period, nitrogen and lime-requirement determinations were made on samples of soil from the rotation plots. For these determinations composite samples were taken by making a number of borings to a depth of 6⅔ inches. The samples were thoroughly mixed and after being dried were passed through a 2-mm. sieve to remove coarse material.

In most cases the soils show a gradual decrease in lime requirement as the amount of limestone was increased. At the end of the first 5-year period, the average lime requirement for the check plots was close to 1200 pounds per acre, while for the plots that received the 2 tons of limestone per acre it was less than 500 pounds. In 1917, at the end of the second 5-year period, the average for the check plots was about 1300 pounds per acre, whereas those plots that received 2 tons of limestone per acre were either alkaline or had a low lime requirement. From this it would appear that the 2-ton application was quite sufficient for a period of 5 years; indeed, where only 1 ton was used the soil had a low lime requirement at the end of the 5 years.

There does not appear to be very much difference in neutralizing power between the calcium limestone and the magnesian limestone.

TABLE 14

Lime requirement and percentage of nitrogen of soils from plots that have received different quantities of lime, 1908–1917

PLOT	LIMESTONE TREATMENT	LIME (CaO) REQUIREMENT PER 2,000,000 POUNDS OF SOIL		NITROGEN	
		1912*	1917†	1912	1917
	Rotation 1—General farm crops				
		lbs	*lbs*	*per cent*	*per cent*
21	Nothing	1200	1200	0 077	0 0794
22	0 5 ton $CaCO_3$ per acre	1000	600	0 075	0 0798
23	1 0 ton $CaCO_3$ per acre .	600	400	0 075	0 0791
24	2 0 ton $CaCO_3$ per acre	000	alk	0 081	0 0901
25	0 5 ton $CaCO_3MgCO_3$ per acre	600	400	0 091	0 0959
26	1 0 ton $CaCO_3MgCO_3$ per acre	700	400	0 093	0 1015
27	2 0 ton $CaCO_3MgCO_3$ per acre	000	alk	0 092	0 1010
	Rotation 2—General farm crops and potatoes				
28	Nothing	800	1400	0 094	0 0994
29	0 5 ton $CaCO_3$ per acre	800	1000	0 097	0 1019
30	1 0 ton $CaCO_3$ per acre	600	400	0 094	0 0931
31	2 0 ton $CaCO_3$ per acre	100	alk.	0 098	0 1041
32	0 5 ton $CaCO_3MgCO_3$ per acre	700	400	0 093	0 1025
33	1 0 ton $CaCO_3MgCO_3$ per acre	400	alk	0 091	0 0954
34	2 0 ton $CaCO_3MgCO_3$ per acre	300	200	0 089	0 0966
	Rotation 3—Corn potatoes, market garden crops				
35	Nothing	1100	1200	0 093	0 0921
36	0 5 ton $CaCO_3$ per acre	800	1000	0 094	0 0912
37	1 0 ton $CaCO_3$ per acre	600	800	0 081	0 0837
38	2 0 ton $CaCO_3$ per acre	400	alk	0 075	0 0808
39	0 5 ton $CaCO_3MgCO_3$ per acre	1100	1000	0 077	0 0800
40	1 0 ton $CaCO_3MgCO_3$ per acre	700	400	0 080	0 0837
41	2 0 ton $CaCO_3MgCO_3$ per acre	500	400	0 082	0 0800
	Rotation 4—Forage crops				
42	Nothing	1200	1400	0 084	0 0899
43	0.5 ton $CaCO_3$ per acre	1100	1200	0 095	0 0998
44	1 0 ton $CaCO_3$ per acre	700	800	0 099	0 1012
45	2.0 ton $CaCO_3$ per acre	600	400	0 089	0 0864
46	0 5 ton $CaCO_3MgCO_3$ per acre	1100	1200	0 079	0 0773
47	1 0 ton $CaCO_3MgCO_3$ per acre	500	400	0 067	0 0679
48	2.0 ton $CaCO_3MgCO_3$ per acre	300	alk	0 098	0 0652

* Limestone applied in 1908.

† Limestone applied in 1913

Percentage of nitrogen in soils

Nitrogen was determined by the Kjeldahl method and in most cases the results reported are an average of four or more carefully made determinations.

It will be recalled that in Part I of this paper, it was pointed out that with the 5-year rotation of non-legumes the limed section was poorer in nitrogen than the unlimed section at the end of both the first and the second 5-year periods. Furthermore, it was shown that the crops on the limed section were only slightly larger than those on the unlimed section. In other words, the lime actually resulted in a considerable dissipation or loss of nitrogen over and above any loss shown by the unlimed plots.

From the figures shown in table 14, it is evident that the lime treatment has not generally resulted in a depletion of the soil nitrogen as compared with the check, or no-lime plot. This may be more clearly shown by comparing the percentage of nitrogen in samples of soil from the check plot for each rotation, with the average percentage of nitrogen in samples from the six lime-treated plots for that rotation.

The figures are as follows·

		1912	1917
		per cent	*per cent*
Rotation 1	Check (no. 21)	0 077	0 079
	Average, no. 22 to 27	0 084	0 091
Rotation 2	Check (no. 28)	0 094	0 099
	Average, no. 29 to 34	0 094	0 099
Rotation 3	Check (no. 35)	0 093	0 092
	Average, no. 36 to 41	0 082	0 083
Rotation 4	Check	0 084	0 090
	Average, no 43 to 48	0 088	0 083

It will be noted that for rotation 1 the lime-treated plots, on an average, show a higher nitrogen content than the check plots; for rotation 2 the average percentage for the limed plots is the same as for the check plot. For rotation 3, the average for the lime-treated plot is about 0.01 per cent less than the percentage for the check plot. For rotation 4 the difference is slightly in favor of the lime-treated plots in 1912 and the check plot in 1917. It will be noted that the figures for 1917 run very close to those for 1912 in both cases.

The lime-treated plots show the greatest gain during the 5 years in rotation 1, and the most loss in rotation 4.

Certainly in this case it can not be said that the lime treatment has been a failure. It has, with only few exceptions, resulted in increased yields, which in several instances have been large, and in addition the nitrogen content of the soil has been fairly well maintained. In the meantime fair crops have been taken from the land. The oats crops, it must be admitted, have been small, partly because of loss from shattering in the field. The potato crops were not fully successful, because the land is not well adapted to potatoes. With these exceptions, and taking the crops for the 10-year period, the yields have probably been above the average for the locality.

It should be pointed out that these results have been secured without the use of farm manure and with only a very moderate amount of commercial nitrogenous fertilizer. Minerals have been applied in generous amounts in order that a deficiency of these might not become a limiting factor.

From the data presented, it is evident that the limestone which was applied has been an important factor in increasing the yields on the rotation plots described here, but it should be remembered that this effect has been more indirect than direct. Its presence in the soil has favored those organisms which associate themselves with the leguminous plants and has thus enabled those plants to get from the air a larger supply of nitrogen than was obtained by plants grown where the conditions are unfavorable for the nitrogen-fixing organisms.

SUMMARY

Four 5-year rotations were carried out on a loam soil. In each rotation there were seven plots; one unlimed and one each that received 1000, 2000 and 4000 pounds per acre of calcium limestone, and one each that received like amounts of magnesian limestone.

In each rotation legume crops were introduced either as one of the main crops or as a green-manure crop between the main crops.

Two 5-year periods for each rotation have been completed.

Acid phosphate and muriate of potash were used in liberal amounts. Light applications of commercial nitrogenous fertilizers were made. No farm manure was used.

During the 10 years the limed plots, with only slight exception, have yielded distinctly larger crops and more total nitrogen than the unlimed plots.

In most cases, the yields were larger with the 2000 and 4000-pound applications than with the 1000-pound application. The two forms of limestone gave results which are quite similar. There appears to be a slight difference in favor of the magnesian limestone.

In the majority of cases, the percentage of nitrogen was higher in crops from the limed than from the unlimed plots.

The use of lime thus resulted in a gain in both quantity of the crop produced and also in the quality.

Analyses of the soil show, in a number of cases, a higher nitrogen content for limed than for unlimed plots; in other cases, the two are about on a level as to nitrogen content and in a few cases, there has apparently been a depletion of the nitrogen in the limed plots.

This work, taken in connection with the work presented in the preceding paper, would seem to indicate that in using lime emphasis should be laid on its power to make conditions favorable for the organisms associated with leguminous crops rather than on its power simply to neutralize soil acids. In other words, its value is more indirect than direct.

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COLORIMETRIC DETERMINATION OF HYDROGEN-ION CONCENTRATION WITHOUT BUFFER MIXTURES, WITH ESPECIAL REFERENCE TO SOILS

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Although it was appreciated at least as early as 1903 (9) that colorimetric measurements of hydrogen-ion concentration might be based upon the properties of the indicators themselves, without involving the use of solutions of known hydrogen-ion concentration--the so-called "buffer mixtures"—no body of data has yet been secured which can be relied upon to furnish a good, if only approximate, system. At present the various indicators are used in connection with buffer mixtures, though the work of Barnett and Chapman (2) makes the use of buffer mixtures unnecessary for much of the range covered by the indicator phenolsulfonphthalein. These investigators use the indicator in a procedure almost identical with that to be recommended in this paper, after having standardized their technique without reference to dissociation curve or constant.

There are available sufficient data from which tables might be calculated for a system for the approximate determination of hydrogen-ion concentration by means of certain of the modern indicators and without the use of buffer mixtures; but evidence has not hitherto been published showing whether such calculations would be justified. This would depend upon whether or not the behavior of these indicators conforms sufficiently well to the equation one would wish to apply, and whether the technical procedures, which it is convenient to use, are free from objection.

The writer has tested these points, determining the apparent dissociation constant and the form of the apparent dissociation curve by simple methods, such as are serviceable in routine, for enough of the indicators selected by Clark and Lubs (4, 6) to cover the range between the hydrogen-ion exponents 3.2 and 9.7. It was the intention to carry out the study to a higher degree of precision, as some of the indicators would repay this, but recent conditions put a stop to the work; and the results, being already of sufficient precision for the ordinary purposes of the writer, are put forward for the convenience of others. A method is presented, the necessary data for which are contained in a single table (table 7). The method should be of especial use in orienting experiments, or in occasional experiments involving hydrogen-ion exponent measurements, either where it is unnecessary to push to the highest degree of precision obtainable, or where the investigator may be content to carry out his measurements to his limit of precision and to record his results in such a form that they may be more closely interpreted when a more precise study of the indicators shall have been completed.[1]

All results have been expressed in terms of Sörensen's hydrogen-ion exponent, instead of the hydrogen-ion concentration itself. The exponent is merely the common logarithm of the reciprocal of the concentration, when the concentration is given in gram-ions per liter in the usual way. As a reaction becomes more alkaline (or less acid) the hydrogen-ion concentration decreases, but the exponent increases, passing through the value 7.0 (at about 20–25°) at physico-chemical neutrality.

PRINCIPLE OF THE METHOD

The theory underlying the colorimetric determination of the hydrogen-ion exponent without buffer mixtures is in short the following. At any given hydrogen-ion exponent the condition of a dissolved indicator is fixed, a certain definite percentage being in the acid form and the rest being in the alkaline form. We may assume that light is absorbed independently by the two forms of the indicator, and hence that the absorption, and in consequence of this the residual color emerging, will be the same whether the two forms are present together in the same solution or whether the forms are separated for convenience in two different vessels and the light passes through one vessel after the other. Therefore, if we know what these percentages are for a given indicator in a given buffer mixture, we can imitate the color shown in the buffer mixture by dividing the indicator in the proper proportion between two vessels, and putting part of it into the acid form with excess of acid, and the rest into the alkaline form with excess of alkali.

For an indicator which acts like a monobasic acid, the percentages in the alkaline and in the acid form should be related to the hydrogen-ion exponent which produces the percentage relation in accordance with the equation

$$\mathrm{pH} = k + \log \frac{\text{(alkaline form)}}{\text{(acid form)}}$$

where pH is the hydrogen-ion exponent of a given solution, k is the apparent dissociation constant of the indicator expressed as a hydrogen-ion exponent, and the ratio (of which the common logarithm is taken) is the ratio existing between the concentrations of the alkaline and the acid forms.[3] The ratio is equivalent to what will later be called the "drop-ratio," and another form of the equation is therefore

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[1] For this purpose the room temperature and the particular indicator used should be stated. Variations of temperature are usually disregarded, and buffer mixtures calibrated at 18° or 20° are used at higher temperatures without any statement as to what the temperature is. In using the method presented here the errors involved in disregarding temperatures may not be the same as if buffer mixtures are used.

$$\text{pH} = k + \log \text{"drop-ratio."}$$

If 5 drops of indicator solution are placed in a test-tube in dilute alkali and 5 drops placed in another test-tube in dilute acid, and compared with various buffer mixtures containing 10 drops of indicator solution until the color seen on looking through one buffer mixture is the same as that seen on looking through the two 5-drop tubes, the volume of all the tubes having been made the same, the drop-ratio is equal to 1; and since the log of 1 is 0, the hydrogen-ion exponent of the buffer mixture is equal to k, the apparent dissociation constant of the indicator expressed as an exponent. This is the principle used by Salm (16) in determining the affinity constants in some indicators.

How well the theoretical equation applies in the case of any particular indicator must be determined by experiment. It is to be noted that if the indicator is a monacid base it will act like a monobasic acid, as far as the equation is concerned. Also, the indicator, though known to be dibasic, may nevertheless act within a limited range of hydrogen-ion exponent as a monobasic acid; namely, if the two dissociation constants (representing the two stages of dissociation) are far enough apart on the exponent scale. This appears to be the case with the sulfonphthaleins (6, 12). In the case of phenolphthalein (which is not a sulfonphthalein) the equation cannot apply, as is well known from the work of Acree (1) and others. If the data given by Rosenstein (17) are used to plot a curve with pH and the percentage transformation into alkaline form as the variables, the curve is found to be a straight line as far as the measurements extend. The percentage of transformation increases faster with the increase of the exponent than the theoretical equation given above demands.

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[3] The equation may easily be derived (by taking the logarithms of both sides and changing signs) from the following one, which is the general equation corresponding to a particular one given by Stieglitz (19), his equation 7' page 1122.

$$H^{\times} = k\,\frac{(\text{acid form})}{(\text{alkaline form})}$$

where the parentheses indicate concentrations. Stieglitz showed that the equation in this form takes account of any tautomeric changes concerned in the change of color, and it is easy to see from his discussion that the form of the equation is the same whether the indicator is a weak acid or a weak base, provided that it behaves within the range of color change under consideration as a monobasic acid or a monacid base. The constant k includes the affinity constant of the tautomeric change as well as the dissociation constant of the acid or base, and if the indicator is a base it includes also the dissociation constant of water. The constant k has therefore been called the apparent [Noyes (16)] or total (Acree) dissociation constant.

Tizard (20) studied the color change of methyl red, using a colorimeter in yellow light, and thus measuring only the change of intensity of the red color. The yellow alkaline color is not apparent under such illumination. He found the indicator to be dibasic, and the two dissociation constants to be near enough together to cause some interference. It does not follow, however, that the theoretical equation given above may not be found to hold, and, conversely, a verification of the equation does not prove the indicator to be strictly "monobasic." As a matter of fact, as will be shown later, the data of Tizard can be used to verify the equation. At higher acidities than those included in the useful range, the color was found to diminish, and it would appear necessary, in more precise work than that contemplated by the use of the method here presented, to regulate with care the strength of the acid solution used to develop the full acid color of this indicator. No difficulty was actually experienced in the present work, and some experiments, made with the use of $0.05M$ buffer mixtures, failed to discover a significant diminution at the same points found by Tizard.

CALCULATIONS BASED ON THE THEORETICAL EQUATION

By means of the technique to be described, the per cent transformation into the alkaline form may be determined in steps of 10 per cent. Table 1 is useful in applying the equation.

Once the equation is shown to be valid for a particular indicator, a table may be made out for the indicator similar to table 7 (to be given later) by adding to each log value the value of the dissociation constant expressed as an exponent, paying attention to the fact that the first five log values are negative numbers (and are not therefore technically logarithms). The resulting figures give then the exponents corresponding to the various drop-ratios. The intervals of the system are about 0.2 pH. If a less precise system is desired for special purposes a 5-drop system may be used instead of the 10-drop system described below, and the desired table may be prepared by simple omission of unnecessary values. Such a system would provide intervals of 0.4 pH and would require indicator solutions of double the strength specified in table 7.

EXPERIMENTS ON WHICH THE METHOD IS BASED

In the study of each indicator a comparator was used which differed from that shown in figure 1 in having only four holes for test-tubes instead of six. Uniform tubes 1.5 cm. in external diameter and 15 cm. long were used. A pair of tubes was prepared containing together 10 drops of indicator solution, the 10 drops being distributed in the two tubes in one of the drop-ratios, 1 : 9, 2 : 8, 3 : 7, and so on, the contents of one tube of the pair being made alkaline and the contents of the other, acid. Each pair of tubes was placed in turn in one side of the comparator and in the other side were placed a tube of water (in the back) and a tube containing 10 drops of the indicator solution in one of the buffer mixtures studied by Clark and Lubs (5). The tubes were nearly uniform, and the volume was made the same in all the tubes, usually 5 to 6 cc., distilled water being added for this purpose to the tubes of the pair. Various buffer tubes were tried until a match was obtained. If the color seen through the pair of tubes fell in an interval between two buffer tubes a quick estimate was made of the fraction of the interval, and this fraction was recorded.

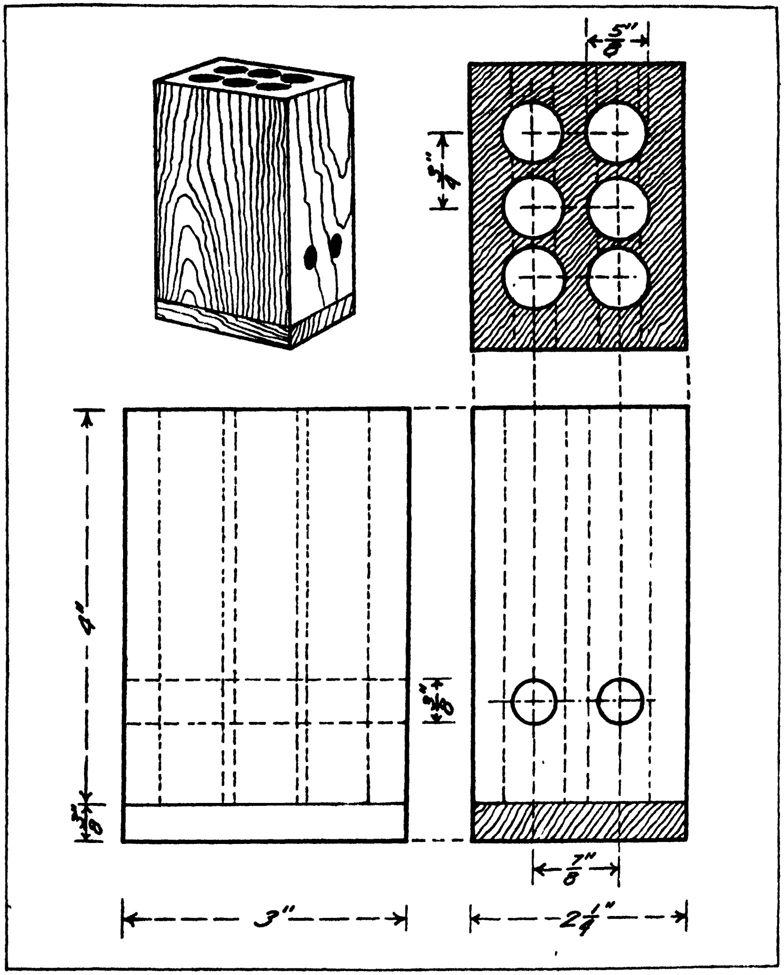

Fig. 1. Comparator for Hydrogen-ion Exponent Measurements

After the measurements were finished the results were calculated from the determined hydrogen-ion exponent values of the buffer solutions. In this way the pairs of tubes of the various ratios were treated as if each pair had been a single solution of unknown hydrogen-ion concentration, and its hydrogen-ion exponent measured colorimetrically. The result may then be termed the hydrogen-ion exponent value of the pair.

A few pairs of tubes were also studied containing a total of 5 drops in the same volume of solution, and others containing a total of 20 drops. In this way the indicators were studied at all concentrations which could be used with test-tubes of about the given dimensions in the given procedure. It was not to be expected that the dissociation constant could show perceptible change with changes of 100 per cent in concentration, since the indicator was in very dilute solution in any case, from 0.00001 to 0.00002 molecular. When alcoholic solutions were used an effect was sometimes seen when the larger quantities of indicator solution were added. This was probably due to change in the nature of the solvent, the concentration of alcohol increasing to about 4 to 6 per cent when 20 drops were used.

TABLE 1

Logarithm values for the various drop-ratios

	1.9	1 5:8 5	2 8	3.7	4 6	5 5	6.4	7:3	8:2	8 5:1.5	9:1
Log value	−0 95	−0 75	−0 60	−0 37	−0 18	0	0 18	0 37	0 60	0 75	0 95
Approximate log value	−0 95	−0 75	−0 6	−0.4	−0.2	0	0 2	0 4	0 6	0.75	0 95

There were at hand a series of buffer mixtures somewhat old. In order to reduce the effect of the personal equation these solutions, which were no longer exact, were used in preference to fresh ones, and the hydrogen-ion exponent of each in turn was determined at practically the same time and at nearly the same room temperature at which they were used. The hydrogen-ion measurements were made in the Clark electrode vessel (3) with mechanical shaking in a well insulated box. The automatic temperature regulation was not used; and the temperature of the box was about that of the room but was far more constant, varying hardly a degree per day. As reference electrode a saturated potassium chloride, calomel, electrode was used and the hydrogen-ion exponents were calculated from the figures given by Michaelis (15). This electrode had been in use for about three years and had been frequently checked with satisfactory results against Clark and Lubs' 0.05*M* acid potassium phthalate solution (5) and Michaelis' standard acetate solution (15). At about 25°, Clark and Lubs' value for 20°, 3.97, was closely checked; at 30° the value found by the above method of reference was about 4.01. In the case of the standard acetate the results were always about 0.4

to 0.5 millivolt (0.01 pH) higher than those given by Michaelis; this being due possibly to the fact that the Clark electrode vessel gives a more certain equilibrium after 5 minutes' shaking than Michaelis seems to have attained with his hydrogen electrode in a considerably longer time. He states that 30 minutes or longer may be required in the case of standard acetate solution, which would seem from his account to take a longer time to reach equilibrium in his apparatus than many other solutions.

In the use of the Clark electrode vessel the vessel was first flooded with electrolytic hydrogen (after filling with water or the solution, unless the air was known to be displaced by previous determinations), and then washed with the fluid; then the fluid admitted, and the vessel rocked for 5 minutes. Hydrogen was not bubbled through the liquid, this procedure not being necessary except in very precise work; and in fact being suitable only for solutions containing no volatile acids or bases (unless the hydrogen is suitably washed before entering the electrode vessel.) It was noted that, because of the volatility of acetic acid, successive determinations of the standard acetate mixture did not check unless the portion of the fluid remaining in the test-tube portion of the vessel was discarded. Such portions evidently lost acetic acid during the 5 minutes' shaking. A small sheet platinum electrode was used, which was coated freshly every day with palladium black and subsequently treated in the usual manner, as described elsewhere (10).

The results of the measurements of the hydrogen-ion exponent value of the various pairs of tubes having drop-ratios 1 : 9, 2 : 8, etc., are given in tables 2 and 3. For the calibration of a system for measurement of hydrogen-ion exponent it is better not to use these individual figures, as they contain experimental errors, but to smooth out the errors by means of the theoretical equation, provided the data are well represented by it. To examine this the values of the constants were calculated by means of the equation and are summarized in table 4. In tables 2, 3 and 4, parentheses are used to enclose figures affected by unusual uncertainties of measurement. In some cases there is an evident effect on the constant of the use of 20 drops of alcoholic indicator solution. Aside from these cases, the apparent dissociation constant is really constant within the experimental error of the color comparisons. This error varies of course with the different indicators, being relatively great with bromphenol blue and bromcresol purple, and small with the red indicators, especially phenol red. The question is of course left open whether or not any inexactness of the equation might be established if means were taken to secure more precise color estimations. It is clear that the equation is entirely suitable for smoothing out the accidental errors.

The results will now be taken up further with each indicator in turn, including some results not summarized in the tables.

Bromphenol blue (Tetrabromo-phenolsulfonphthalein; M. W.,[3] 670). This indicator, because of its dichromatism, sometimes gives trouble with turbid solutions, as was discussed by Clark and Lubs, who recommended (6) a screened light (yellow) for use when difficulty is experienced in daylight. All the figures reported in tables 2 and 3 were obtained by daylight measurements, but this indicator, as well as some of the others, was also studied in the yellow screened light. The constants obtained were not quite the same, being 4.00 for the aqueous indicator solution, and 3.99 for the alcoholic indicator solution (10-drop series), as against 4.06 and 4.04 for the daylight measurements.

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[3] Molecular weight.

TABLE 2

Hydrogen-ion exponent values found for the various pairs. Indicators used in water solution

DROP-RATIO	INDICATOR					
	Brom-phenol blue	Methyl red	Brom-cresol purple	Phenol red	Cresol red	Thymol blue (alkaline range
1.9	3 20	4 20	5 36	6 83	6 94	(8 01)
2·8	3 38	4 35	5 61	7.09	7 44	8 27
3 7	3 69	4.65	5.79	7.34	7.67	8 51
4.6	3 83	4 83	6 10	7 52	7 92	8 63
5.5	4 02	4 96	6 21	7 74	8 08	8 76
6.4	4 21	5 12	6 40	7 92	8 30	9 02
7 3	4 30	5 32	6 72	8 11	8 43	9 10
8 2	4 73	5 56	6 89	8 32	8 67	9 42
9.1		(5 85)	7 09	(8.59)	8 84	(9 57)
1.4		4 38				
2 3		4 77		7 56		
3 2	4 35			7 85		
4·1	(4 8)			8 34	8 71	
2·18	3 15		5 31	6 82	7 06	7 87
4 16	3 40		5 56	7 09	(7 41)	8 23
6 14			5 82	7.29	7 67	8 47
8·12			5 96			8 69
10·10				7 72		8 79
12.8						8 97
14·6				(8.00)	8 45	9 16
16.4		5 50				9 37
18 2		5 96				9 70
Room temperature	31°	30°	30°	29°	24°	24°
k	4 06	4 96	6 26	7 72	8 08	8 82
Indicator solution used (per cent)	0 008	0 003	0 012	0 004	0 008	0 008

The discrepancies, which of course cannot be due to a real change of the dissociation constant, are in all cases within the possible error of interpolation, and are no doubt entirely due to this error, as in certain cases the comparison was repeated with the error of interpolation halved by the use of buffer mixtures only 0.1 pH apart, and the discrepancy was in those cases reduced correspondingly. The discrepancy was apparently due to the use of the screened light in a room not otherwise darkened, and also to certain relative positions of the screened light and the windows. If measurements are to be made with the screened light in a room which cannot well be darkened, the screened light should be so placed that white light does not enter the eye from one side or the other. Because of these considerations, the values found for daylight have been used in the preparation of tables for use either in daylight or in the yellow light. Similar discrepancies due to the same cause were noted with some of the other indicators.

TABLE 3

Hydrogen-ion exponent values found for the various pairs. Indicators used in alcoholic solution

DROP-RATIO	INDICATOR				
	Brom-phenol blue	Methyl red	Brom-cresol purple	Brom-thymol blue	Brom-thymol blue
1.9	3 38	4 00	(5 23)	6 10	(6 10)
2:8	3 40	4 42	5 60	6 38	(6 35)
3·7	3 67	4 69	5 82	6 68	6 66
4·6	3 78	4 85	5 82	6 86	6 85
5.5	4 04	5 01	6 12	7 04	7 06
6 4	4 21	5 19	6 29	7 23	7 29
7.3	4 44	5 34	6.48	7 47	7 47
8.2	(4 74)	5 56	6 67	7 79	7 70
9 1		5 82	(7 13)	7 98	
1 4		4 44			
2:3					
3:2			6 44		
4·1			(7 13)		
2:18	3 10		5 14		6 12
4·16	3 37		5 42		
6·14		4 64	5 66		6 72
8:12			5 85		
10:10	3 90		6 10		7 02
12:8			6 27		
14:6	4 28	5 34	6 44		
16:4	4.44		6 76		7 57
18:2	4.69	5 88	(7 02)		7 87
Room temperature.	29°	27°	30°	25°	27°
Indicator solution per cent .	0 008	0 008	0 024	0 03	0 015
Alcohol (per cent) in indicator solution .	95	60	95	60	60

A few measurements were made toward determining the temperature coefficients, using for this purpose fresh phthalate buffer mixtures, spaced at 0.1 pH intervals. They were made up and checked at 20°. Measurements of the temperature coefficients of the buffers were not made. The aqueous indicator solution was used and the pair having the drop-ratio 7 : 3 was studied. The buffer mixtures were kept in a water bath regulated at a given temperature and were removed an instant at a time to permit quick measurement in the comparator of the hydrogen-ion exponent value of the 7:3 pair. No certain difference in the value could be seen on varying the temperature in several steps from 15 to 50°. The values could be estimated only within 0.1 pH. This result does not mean that the temperature coefficient is necessarily negligible, since the change of hydrogen-ion exponent of the buffers might possibly conceal a small effect.

TABLE 4

Calculated values of the apparent dissociation constants

DROP-RATIO	BROMPHENOL BLUE		METHYL RED		BROMCRESOL PURPLE		BROMTHYMOL BLUE		PHENOL RED	CRESOL RED	THYMOL BLUE
	W*	A†	W	A	W	A	A	A	W	W	W
1·9	4 15	4 33	5 15	4 95	6 31	(6 18)	7 05	7 05	7 78	7 89	(8 96)
2:8	3 98	4 00	4 95	5 02	6 21	6 20	6 98	6 95	7 69	8 04	8 87
3:7	4 06	4 04	5 02	5 06	6 16	6 19	7 05	7 03	7 71	8 04	8.88
4:6	4 01	3 96	5.01	5 03	6 28	6 00	7 04	7 03	7 70	8 10	8 81
5:5	4 02	4 04	4 96	5 01	6 21	6 12	7 04	7 06	7 74	8 08	8 76
6.4	4 03	4 03	4 94	5 01	6 22	6 11	7 05	7 11	7 74	8 12	8 84
7:3	3 93	4 07	4 95	4 97	6 35	6 11	7 10	7 10	7 74	8 06	8 73
8:2	4 07	(4 14)	4 96	4 96	6 29	6 07	7 19	7 10	7 72	8 07	8 82
9:1			(4 90)	4 87	6 14	(6 18)	7 03		(7 64)	7 89	(8 62)
1.4			4 98								
2:3			4 95						7 74		
3 2	4 17					6 26			7 67		
4:1	(4 2)					(6 53)			7 74	8 11	
2:18	4 10	4 05			6 26	6 09		7 07	7 77	8 01	8 82
4:16	4 00	3 97			6 16	6 02			7 69	(8 01)	8 83
6 14				5 01	6 19	6 03		7 09	7 66	8 04	8 84
8 12					6 14	6 03					8 87
10.10		3 90				6 10		7 02	7 72		8 79
12·8						6 09					8.79
14 6		3 91		4 97		6 07			(7.63)	8.08	8.79
16·4		3 84	4 90			6 16		6 97			8 77
18 2		3 94	5 01	4 93		(6 07)		6 92			8 75
Constant	4 06		4 96	4 99	6 26				7 72	8 08	8 82
Constant, 10-drop series		4 04				6 14	7 06	7 05			
Constant, 20-drop series		3 94				6 07		7 01			

* W—Water indicator solution.

† A—Alcohol indicator solution.

Methyl red (4'-dimethylamidoazobenzene-2-carboxylic acid; M. W., 269). There seemed to be no important influence of the alcohol when 10 drops of the alcoholic indicator solution were used. The pipette delivered 48 drops per cubic centimeter. After addition of 10 drops to the buffers the volume was 6.5 cc., and the concentration of absolute alcohol was therefore about 2 per cent.

The temperature coefficient was studied as in the case of bromophenol blue, with the same buffer mixtures. The aqueous indicator solution was used, and the pair having the drop-ratio 2 : 8 was studied. The dissociation constants were calculated by adding 0.60 to the hydrogen-ion exponent values found for the 2 : 8 pair. It should be noted that the constants are not corrected for the change of hydrogen-ion exponent of the buffer mixtures with temperature, and the true variation of the constant with temperature is probably a little greater than would appear from these results. Table 5 gives the results. The value for 18° is one calculated from the data of Tizard.

TABLE 5

Apparent dissociation constant (uncorrected) of methyl red at different temperatures

TEMPERATURE CENTIGRADE	14.5°	18 0°	20 0°	29.5°	40.0°	49.5°
Constant..	5 13	5 11	5 10	5 05	4 98	4 93

Tizard arrived at the constant 4.98, but the mode of calculation is not suited to present purposes. He came to the conclusion that at most only 93 per cent of the molecules could actually be brought into the red form by an excess of acid and took this into account in the calculation, basing the percentages of color transformed on the theoretical maximal transformation. Now in the present method we must take the actually realizable maximal redness, as obtained by excess (not too great) of acid, as 100 per cent. The writer has therefore recalculated the data of Tizard, taking the maximal redness observed by him as 100 per cent as the basis for calculating the percentage transformed. The results are given in table 6. His results for the more alkaline portion of the range are omitted. The extreme values of the constant given in table 6 deviate from the others, but within the range 10 to 90 per cent transformation the constancy is very good. Neglecting the two extremes, the mean value is 5.11.

TABLE 6

Apparent dissociation constant for methyl red at 18°, calculated from the data of Tizard.

	PER CENT IN THE ACID FORM											
	8.75	11 7	14.3	19 0	22 9	30 6	39 8	47.6	57.9	72 6	86 0	98.5
Apparent constant... . ..	5 25	5 17	5 12	5 12	5 10	5 09	5 09	5 10	5 11	5 09	5 15	5 86

Propyl red (Dipropylamidoazobenzene-2-carboxylic acid). This indicator has given very good results as used in buffer mixtures, but the colors of the standards are apt to fade prematurely. When the attempt was made to measure the constant the colors in the buffers faded before the measurements could be finished. The equation did not seem to apply at all, but it was observed that after the colors had faded (in the more acid tubes of the buffers) the equation seemed to fit better. This gives a hint that a tautomeric change, not instantaneous, is involved, but unfortunately this was not followed up at the time. If this is true, the constant would be about 5.28 at 30°. This indicator is not included in the present method.

Bromcresol purple (Dibromo-*o*-cresolsulfonphthalein; M. W. 540). When the alcoholic indicator solution was used a difference in the dissociation constant was noted of 0.12 in the 10-drop series, with a further difference of 0.07 in the 20-drop series. No attempt was made to measure the exponent of the buffer mixtures after addition of alcohol.

TABLE 7

Data for the determination of hydrogen-ion exponent

DROP-RATIO	HYDROGEN-ION EXPONENT FOR EACH PAIR OF TUBES						
	Brom-phenol blue	Methyl red	Brom-cresol purple	Brom-thymol blue	Phenol red	Cresol red	Thymol blue
1:9	3.1	4.05	5.3	6.15	6.75	7.15	7.85
(1.5:8.5)	3.3	4.25	5.5	6.35	6.95	7.35	8.05
2:8	3.5	4.4	5.7	6.5	7.1	7.5	8.2
3:7	3.7	4.6	5.9	6.7	7.3	7.7	8.4
4:6	3.9	4.8	6.1	6.9	7.5	7.9	8.6
5:5	4.1	5.0	6.3	7.1	7.7	8.1	8.8
6:4	4.3	5.2	6.5	7.3	7.9	8.3	9.0
7:3	4.5	5.4	·5.7	7.5	8.1	8.5	9.2
8:2	4.7	5.6	6.9	7.7	8.3	8.7	9.4
(8.5:1.5)	4.8	5.75	7.0	7.85	8.45	8.85	9.55
9:1	5.0	5.95	7.2	8.05	8.65	9.05	9.75
Per cent in indicator solution	0.008	0.008	0.012	0.008	0.004	0.008	0.008
Cubic centimeters of 0.1 N NaOH per 0.1 gm. portion	1.64		2.78	1.77	3.10	2.88	2.38
Produce acid color with	0.05N HCl	0.05N HCl	0.05N HCl	0.05N HCl	0.05N HCl	2% H_2KPO_4	2% H_2KPO_4 or H_2O
Quantity of acid used to produce acid color .	1 cc.	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop

Bromthymol blue (Dibromo-thymolsulfonphthalein; M. W. 624). This indicator was studied only with its solution in alcohol. When 20 drops were used the constant was different by 0.04, not a large difference in the case of this indicator, the colors of which are not so favorable for exact comparisons as most of the other indicators. The water solution of the monosodium salt will be recommended, and since the constant was found to be 7.01 with 20 drops of alcoholic solution, and 7.05 with 10 drops of the same, we may properly round off the latter value to 7.1, as has been done in table 7.

In a committee report of the Society of American Bacteriologists (8) tests were reported of the value of six pairs according to the technique of Barnett and Chapman. The technique differed only in that the volume was not kept constant in all the pairs (this should not be of great significance). The values for the ratios from 1 : 9 to 6 : 4, inclusive, were respectively 6.2, 6.4, 6.7, 6.9, 7.1 and 7.3, essentially the same values as reported here.

Phenol red. (Phenolsulfonphthalein, M. W. 354). This indicator is the one studied by Barnett and Chapman, from the ratio 1:9 to the ratio 6:4, inclusive. Their values were 6.9, 7.2, 7.5, 7.7, 7.9, and 8.1, respectively. Calculation shows that all these values lead to very nearly the same value of the dissociation constant, 7.87. In the report of the bacteriological committee already cited, the values reported for these six ratios were 7.1, 7.3, 7.5, 7.7, 7.8, and 7.9. Calculation of the constant yields values varying considerably, the average value, however, being identical with that given by Barnett and Chapman. It is evident that the use of values for the drop-ratios without smoothing out the experimental errors of the calibration would lead to considerable unnecessary error.

In view of the consistent difference between the writer's values and those of Barnett and Chapman (about 0.2 pH) the writer cooled the buffer mixtures (from 29°) to 23° and determined the values of two pairs, and found that the constant was increased by about 0.05 pH. The hydrogen-ion exponents of the phosphate and borate buffers were determined at 27–28° only. Part of the small discrepancy between the results of Barnett and Chapman and those of the writer may therefore be due to the influence of temperature, either on the dissociation constant of the indicator or on the hydrogen-ion exponents of the buffer mixtures. At present there has been published no system of buffer mixtures which has been calibrated at temperatures higher than 20°. Ordinary differences of room temperature are generally neglected in biological work, though of course such neglect may not always be justified. Because the temperature was not specified and the constants only approximately measured for purposes of illustration, the constants given by Clark and Lubs (6) for the various indicators have not been compared with the present values. A number of them differ by about 0.1 or 0.2 from those of the writer, and in the case of phenol red their value is in agreement with those of that of Barnett and Chapman.

Cresol red is ortho-cresolsulfonphthalein, and has the molecular weight 382.

Thymol blue is thymolsulfonphthalein, and has the molecular weight 463. The changes in strong acid solution were not studied.

PREPARATION OF THE INDICATOR SOLUTIONS

At present the indicators are offered in the market both in the solid form, and as solutions of the monosodium salts. The preparation of phenol red offered for a renal function test, containing carbonate, is unsuitable.

The suitable strengths of solution for the preparation of the color standards are given in table 7. The percentages refer to the weight of the free acid indicator. It is understood that precision is not required in the concentration of indicator solution; however, in the preparation of the monosodium salts ordinary care should be observed in the measurement. The quantities of alkali required to form these salts from the solid, free, indicators are given to 0.01 cc. but it is only required to work as well within a tenth of a cubic centimeter as possible.

Although methyl red can be prepared as a monosodium salt in water solution, this is not advised for measurements of the exponents of soil extracts; the alcoholic solution being preferred for reasons given recently (10). The following directions require an analytical balance. Take 20 mgm. of finely ground indicator, dissolve in 150 cc. of alcohol which has been redistilled over a little alkali, and make up the volume to 250 cc. with distilled water. The other solutions required are all made up in water solution. In all cases use a clean mortar (best of agate) and grind up the indicator with the required quantity of standard sodium hydroxide solution, added in portions, at first only moistening the indicator. The required quantity of 0.1 *N* sodium hydroxide solution for 0.1 gm. of indicator is given for each indicator in table 7. These quantities give 1.1 equivalents for each indicator except for bromcresol purple, for which the quantity gives 1.5 equivalents, the stated number of equivalents being required for solution, according to a personal communication from Dr. H. A. Lubs. We generally use 50 mgm. instead of 0.1 gm., using sodium hydroxide more dilute than 0.1 *N* in order to obtain accurate measurement. Heat may be used to effect solution, but should not be necessary. We reported difficulty in avoiding decomposition of bromcresol purple (10) but have had no trouble since that time and believe that the present technique, which is that given by Clark and Lubs (7), avoids all difficulty.

Some of the preparations of indicators on the market are not quite pure, unchanged starting material apparently being present in some brominated indicators. For the preparation and purification of these indicators reference may be made to the work of Lubs and Clark (13, 14) for all but methyl red; and to that of Tizard for methyl red (20). The papers of Acree and his students contain information on this point, for which see reference no. 22 and literature given there. The following notes, obtained from these sources, may be of interest. Bromphenol blue may be recrystallized from glacial acetic acid or (22) from a mixture of acetic acid and acetone; methyl red and bromcresol purple from boiling toluol; cresol red, from glacial acetic acid; and thymol blue, from alcohol.

If the indicators contain impurities having indicator properties, the fact may be disclosed in use by the impossibility of matching the color of the unknown solution by means of any of the color standards.

PREPARATION AND USE OF THE COLOR STANDARDS

The comparator shown in figure 1 is modified from those used by Hurwitz, Meyer and Ostenberg (11) and by Clark and Lubs (6), the principle of which is the same as that of the comparator of Walpole (21). The dimensions are suitable for test-tubes the external diameter of which is a little less than ⅝ inch, as is the case with those described below. The essentials are that the various holes should lie in the proper planes, as shown by the vertical broken lines. These planes should lie as closely together as possible so that the two optical fields are near together; and the test-tubes should not be too loose in the holes, as otherwise the optical picture may depend somewhat on the position of the tubes in the holes. The view-holes are intentionally smaller than the tube diameter in order to give a homogeneous optical picture, and it is partly for the same reason that the water tubes are inserted. The interior of the comparator is best painted dead black.

Test-tubes of 1.5 cm. external diameter and 15 cm. long are suitable for the comparator illustrated and for the strengths given for the indicator solutions. It is advisable to select from a stock of tubes a sufficient number of uniform tubes by running into each 10 cc. of water and retaining those which are filled nearly to the same height. A variation of 3 or 4 mm. on a height of 8 cm. is permissible. Test-tubes without flanges are preferable. The tubes may be held together in pairs by means of one rubber band per pair, which is wound about the tubes in the form of two figure 8's.

It is convenient to use metal test-tube racks, one for each indicator, each rack holding two rows of tubes, accommodating one tube of each pair in front and one in back. For any desired indicator a set of color standards is prepared by placing from 1 to 9 drops of the indicator solution in the 9 front tubes of the pairs and from 9 to 1 drops in the back row of tubes.[4] A drop of alkali is then added to the tubes in the front row (two drops in the case of thymol blue), sufficient to develop the full alkaline color, and a quantity of acid is added to the tubes of the back row to develop the full acid color without causing a secondary change of color. The quantity of acid to be added varies a little with the nature of the indicator. Table 7 tells the quantity and nature of the acid which can be used without danger of difficulty. If by accident more than one drop is added, no trouble should be caused, and the strengths given need only be approximate. The 0.05 N HCl may be prepared by diluting 1 cc. of concentrated hydrochloric acid (sp. g., 1.19) to 240 cc.; and the alkali, by making up a 0.2 per cent solution of stick sodium hydroxide. The volume is at once made up in all the tubes to a constant height (within about one drop) with distilled water, the height corresponding to 5 cc. A different volume may be chosen if desired. A rubber tube with a pinch-cock serves to deliver the water. In making up the volume it is convenient to hold in the hand a tube containing the chosen volume beside the pair of tubes to be filled. These operations can be carried out rapidly. Investigators may determine for themselves under their working conditions how long the standards may be relied upon to keep. We have found most of them stable for more than two days, and it would appear that no change whatever need be feared during the first day except possibly in the case of cresol red and thymol blue. The ratios given in parentheses in table 7 are secured by the use of a total of 20 drops, distributed 3 drops in one tube of the pair and 17 in the other, and the volume is made double (10 cc.). Such tubes are used in exactly the same way as the others. It will be clear that the ratios 8.5: 1.5, methyl red, and 1.5: 8.5, bromcresol purple are necessary if it is desired to make measurements around the exponent 5.6 as precise as at other points, but the other 20-drop pairs are seldom useful unless some of the indicators are omitted.

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[4] We take the natural precaution of holding the delivery pipette in a vertical and therefore easily reproducible position when adding the indicator drops.

If only a few measurements are to be made, it will be desirable to determine what indicators can be used before preparing the color standards. This is readily done in most cases by treating the unknown solutions with the indicators in turn until one of the indicators shows a partial transformation of color. If material is limited this test (or even the actual measurement) may be made by adding 2 drops of indicator solution to 1 cc. of solution. In some cases it may be necessary to use the comparator and a few color standards in order to make sure whether a given indicator can be used or not. When the necessary standards for the needed indicators have been prepared the solution to be examined is treated with 10 drops of indicator solution, such a quantity of the solution being taken in preference that the mixture rises in the test-tube to the height representing a volume of 5 cc. The test-tube containing the mixture is placed in the comparator and its color compared with the color of the standards, any natural color or turbidity of the solution being balanced optically, as shown below. Various color standards are tried, one pair after another, until the color of the unknown is matched with one of the pairs or is evidently between the colors of two of them. In all cases it must be determined that the color is certainly within the range; thus measurements cannot be made at the point where the ratio is 9: 1 or 1: 9, as the percentage transformation of the indicator is so nearly 100 or zero per cent that the hydrogen-ion exponent might really be far from that represented by such a ratio without the fact being disclosed by a color difference. With turbid solutions it may not always be possible to locate the correct ratio when it lies even at 8: 2 or 2: 8. When the ratio has been found which corresponds to the color of the unknown, the hydrogen-ion exponent is found from table 7. Thus suppose methyl red is the indicator used for such a comparison and the color of the unknown lies between the colors of the standards having the ratio 5: 5 and 6: 4, then the hydrogen-ion exponent is found from the table to be between 5.0 and 5.2, or may be put at 5.1, if the color was about as near one ratio as the other. When the color standards are being tried in succession to determine which pair matches most nearly with the fluid of unknown hydrogen-ion exponent it is advisable to take a simple precaution. If one continues to gaze through the comparator while removing a color standard the color of the tested fluid will appear very strong as soon as the color standard is removed, and this enhancement of color will persist to a noticeable extent when the new color standard is inserted. On this account the change of color standards should always be made without looking through the comparator. It is advisable to make a number of changes of the standards, working rapidly. The eye acquires a temporary sensitiveness of color appreciation.

A good arrangement of the tubes in the comparator for a right-handed operator is the following. Referring to the top view in figure 1, two tubes of water are placed in the two upper left-hand holes and the solution to be examined, treated with indicator, below. The solution without indicator may be placed in the upper right-hand hole, and a pair serving as a color standard in the two lower right-hand holes. It is a natural choice to place the pair so that the color which is dominant is nearer the eye.

The tubes are viewed preferably against the sky. If, in the case of some of the indicators (bromcresol purple, and especially bromphenol blue) a difference of quality appears, due to dichromatism, so that no satisfactory match can be obtained, it will be advisable to view the tubes for the comparison in a yellow light as suggested by Clark and Lubs (6). They use a box containing carbon electric incandescent lamps and the light emerging from the box passes through tracing paper stained yellow by painting it with a mixture of phenolsulfonphthalein (phenol red) (5 cc. of 0.6 per cent solution) and acid phosphate solution (5 cc. of a 3 per cent solution of KH_2PO_4). In using the screened light either white light should be shut off, or at least the arrangement should be such that white light does not enter the eye from one side while viewing the tubes in the yellow light. It is only with very turbid solutions that it is necessary to work with the yellow light.

In using 10 drops of an aqueous indicator solution in a final volume of 5 cc. there is involved a dilution which may in special cases be undesirable. If the solution under examination owes its hydrogen-ion concentration to a strong acid, such a dilution would cause the exponent to be raised by about 0.046, an error not always negligible. This is the greatest error that can be caused by such a dilution when an aqueous indicator solution is used. (We are assuming that 10 drops occupy a volume of 0.5 cc.). If the solution owes its hydrogen-ion concentration to a weak acid the same degree of dilution would cause the exponent to be raised only by about 0.02, while if salts of the weak acid are present the error will be very much smaller. In most biological fluids no error as large as 0.02 is to be expected; with soil extracts it has not yet been determined how nearly the error may approach the maximum, 0.05 pH. If it is desired to reduce the error, each indicator may be used in two strengths, a dilute solution of the strength prescribed above being used for the color standards, and a solution 5 times as concentrated being used for the solution itself, 2 drops being added instead of 10 drops. In the work recently reported in this journal on soils (10) we used 2 drops of the aqueous indicator solutions to 5 cc. of soil extract, except in the case of methyl red, where 4 drops were used. If the dilute alcoholic solution of methyl red is used in soil extracts, the error due to addition of the alcohol in 10 drops is probably negligible but has not yet been shown so. With methyl red a solution 5 times as strong cannot be prepared in 60 per cent alcohol but one may be prepared 2.5 times as strong as the dilute solution, and 4 drops of this may be used in the soil extract.

A NOTE ON CLEAR SOIL EXTRACTS BY THE USE OF COLLOIDAL IRON

That clear soil extracts can be obtained by the use of colloidal iron is probably known, though the writer is unable to adduce any references, no literature search having been made on this point. The well-known principles of colloidal chemistry apply to the case; namely, in the precipitation of the (negative) colloids by the (positive) colloidal iron the reaction is nearly quantitative, but not to an inconvenient degree, so that practically clear solutions can be obtained with some variation in the proportions of the soil and the colloidal iron. If the iron is added in portions, a larger quantity is required, also a slight excess of iron may be caused to disappear by again shaking up the soil with iron.

A commercial "dialyzed iron" was found to contain enough acid to affect somewhat the hydrogen-ion exponent of the extracts obtained by its use. Although the addition of silver nitrate did not cause precipitation (at least immediately), an immediate change was easily visible upon examination by reflected sunlight through the side of the test-tube. On dilution the solution showed a high acidity against methyl red. After removal of the iron by addition of ammonia and boiling, a strong test for the chlorine ion was given with silver nitrate in the presence of dilute nitric acid. The preparation contained 7.5 gm. of solids in 100 cc. Gravimetric analysis showed a ratio, Fe_2O_3:AgCl, equal to 2.9. The variations in the formula for the "oxychloride" of dialyzed iron given by the United States Dispensatory (19th edition), on the authority of Graham, correspond to variations in this ratio of 2.4 to 8.8.

A quantity of the commercial preparation was redialyzed in a thin collodion bag in running distilled water, the velocity of which was increased about the bag by putting it in a tube of only slightly greater diameter. The dialysis continued two days and the ion did not precipitate in this time. Water entered during the dialysis, causing a dilution of about 25 per cent. The redialyzed product was found to have lost but very little of its precipitating power for soils. When it was diluted, two drops to 10 cc., and attempts were made to determine its hydrogen-ion exponent, methyl red gave the exponent 4.8; and bromcresol purple, a very different result, 6.2. Analysis showed the ratio Fe_2O_3: AgCl, equal to 6.4.

This redialyzed iron was used to obtain clear soil extracts for the measurement of hydrogen-ion exponent in the following way. To 30 cc. of distilled water in a wide test-tube, 15 gm. of the air-dry soil were added, the tube closed and violently shaken 50 times. The dialyzed iron solution was added and the whole immediately shaken a few times to mix thoroughly. On standing, if the proper quantity of iron had been added, the soil particles began at once to subside and soon left a perfectly clear extract above, save for a few floating particles around the edge of the surface. If not quite enough iron was added, fine white suspended particles could be seen, which did not interfere with the measurements. Excess of iron, which is shown by the color of the suspended particles, was carefully avoided, as this had already been found to yield results more or less too acid. The clear fluid was drawn off in a pipette well rinsed with distilled water and the hydrogen-ion exponents determined by the technique given in this paper. The results are given in table 8, together with the results which had previously been obtained for the given soils colorimetrically, by the use of a centrifuge (without iron) and buffer mixtures, and also electrometrically. The first seven soils were Caribou loam and the last two Washburn loam.

TABLE 8

Hydrogen-ion exponents measured for some soils by the use of the colloidal iron method of clearing, compared with the results obtained by the usual methods

SOIL	QUANTITY OF IRON SOLUTION ADDED	HYDROGEN-ION EXPONENTS		
		Iron method	Centrifuge method	Electrometric method
1	8 drops	4 55	4 5	4 50
2	8 drops	4 9	4 9	4 64
3	7 drops	4 8	4 8	4.80
4	9 drops	4 95	4 75	4 80
5	6 drops	5 05	5.0	4.90
6	2 cc.	5 0	5 0	5 00
7	2 cc.	5 2	5 0	5 00
8	1 cc.	5 65	5 6	5 68
9	12 drops	5 8	5 7	5 75

In the case of the Caribou and Washburn loams listed in table 8 the extracts prepared with iron gave practically the same results as did those obtained with the centrifuge (and of course without iron). Those obtained with iron are also far clearer than can ordinarily be obtained with a centrifuge of usual power. A heavy red (Susquehanna) clay, not air-dried, suspensions of which could not be cleared sufficiently with a centrifuge to permit any measurement of hydrogen-ion exponent, gave a water-clear extract by the use of a small quantity of iron solution. The use of iron would be of especial use in the case of fresh soils, which are more "colloidal" than after air-drying.

Nevertheless, the writer does not feel justified in proposing such clearing with iron for the measurement of hydrogen-ion exponent, on the ground that other soils, with possibly smaller "buffer action," might possibly be changed by the residual acidity of the colloidal iron used. It is not supposed that colloidal iron can really be prepared entirely free from acid. It is supposed to be a mixture, in varying proportions, of oxychloride and hydrate. The hydrate of the mixture does not, however, show the same solubility relations as does freshly precipitated hydrate. Although the ratio, Fe_2O_3: $AgCl_3$, for a given preparation would serve in some degree to specify the degree of dialysis, it would appear that considerable more work would have to be done on the subject in order completely to specify the properties necessary for obtaining reliable results. It may, however, be well worth while for those who can check the results for different types of soil, either electrometrically or colorimetrically by the use of a centrifuge, to test the applicability of the method for themselves.

SUMMARY

A simple technique has been described in full for the preparation and use of a series of color standards for the colorimetric determination of hydrogen-ion exponent. No buffer mixtures are required. From the method of calibration, however, any salt or protein errors will be the same as if the measurement had been made with the 0.05 *M* buffer mixtures of Clark and Lubs.

Each color standard consists of two test-tubes, one tube containing dilute alkali and the other dilute acid. The tubes contain altogether 10 drops of indicator solution, the ten being divided between the alkaline and the acid tubes in various "drop-ratios."

A table is given (table 7) containing all the necessary data.

The method is based on a study of the nature of the color change of the indicators with change of hydrogen-ion exponent (pH). For all the indicators selected the following equation, which comes from the mass action law, was found to hold within the experimental error of the color readings:

$$pH = k + \log \text{(drop-ratio)}.$$

The indicators studied are from the selection of Clark and Lubs; and the values of the constant k of the equation, good to about 0.1 at 25–30°, are as follows: bromphenol blue, 4.1; methyl red, 5.0; bromcresol purple, 6.3; bromthymol blue, 7.1; phenol red, 7.7; cresol red, 8.1; and thymol blue (alkaline range), 8.8.

Soil extracts were prepared, water-clear, by the use of colloidal iron solution. Measurement of the hydrogen-ion exponents of these extracts (from nine soils only) gave the same results as were obtained by the usual methods. Without further study, however, such use of colloidal iron cannot be recommended for general use.

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THE COMPARATIVE RATE OF DECOMPOSITION OF GREEN AND CURED CLOVER TOPS IN SOIL

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INTRODUCTION

The rate of decomposition of leguminous crops is becoming an extremely important practical question in soil fertility and crop production. The rate of nitrate production is the most important phase of this subject, bearing directly upon the value of the crop for soil improvement either in the green or in the cured condition. Two methods have frequently been employed for measuring the rate of decomposition of organic materials such as occur in nature. One consists in measuring the carbon dioxide production at various intervals; the other is concerned with the determination of nitrate nitrogen at various periods. This latter method is superior in most cases, as it is possible to determine the actual amounts of nitrate nitrogen present, the amount used by the crop, and that lost by leaching. It furnishes data on the nitrogen requirements of the plants at critical periods. The importance of the element nitrogen and the changes occurring in the nitrogen cycle demand more consideration than the changes occurring in the carbon cycle, owing to the solubility, expense and amount of the element nitrogen required for crop growth. There has been no method developed that indicates that there is a true ratio between the amount of carbon oxidized and the amount of nitrogen oxidized. That such a ratio may exist at certain times for some crops cannot be denied, but the fact that non-nitrogenous materials constitute a large proportion of most of the materials that are used for soil enrichment and that the ratio of carbon to nitrogen is constantly changing with maturity, is sufficient evidence to indicate clearly the wisdom of studying the nitrate production in preference to that of carbon dioxide.

Red clover and sweet clover (*Melilotus alba*) are seeded in small grains, usually in the winter or early spring, to be plowed under the following year, either green or after a seed crop has been taken.

It will be evident that any condition which may be found or that can be created that will insure ample nitrate nitrogen from a leguminous crop plowed under as manure for the succeeding crop of that year, will assist materially in increasing crop yields and in eliminating nitrogen as a limiting element.

Furthermore, it will be just as obvious that a retarded nitrate nitrogen production, which fails to supply nitrate at the critical periods in the growth of the crops, but which may supply large amounts later, will tend to result in wasteful losses through leaching, and not infrequently in delayed growth and maturity of the crop. The importance of controlling the rate of nitrate production is such as warrants extended study of this subject.

This paper is a contribution to that phase of the subject of the decomposition of leguminous crops that is concerned with the comparative rate of decomposition of common red clover tops, when employed as a manure, in the green and in the cured condition.

HISTORICAL RÉSUMÉ

Only a few articles have been found in the literature which have a bearing upon the particular phase of the subject under study.[1] Potter and Snyder (3) studied, in one case only, the decomposition of green clover and compared it with the decomposition of some dry clover in another experiment. While a direct comparison is not available, their results indicate a more rapid production of carbon dioxide in soil treated with the green clover than in that treated with air-dried finely-ground clover. This work is in accordance with other experiments in regard to the rapid initial decomposition that takes place with green manures. No study was made of the nitrogen in the materials and therefore the work is not of assistance in estimating the comparative production of nitrates.

Boltz (1) reported two experiments in which green clover incorporated with the soil was compared with green clover allowed to remain on the surface, and found more decomposition had taken place with the clover on the surface than when incorporated with the soil. This experiment furnishes valuable information in connection with fall plowing of crop residues compared with leaving them on the surface. It is rather surprising to find such a large decomposition of carbonaceous material with no apparent loss of nitrogen. The amount of carbon represented as lost with no nitrogen loss indicates clearly a decomposition of non-nitrogenous constituents of the material or only a partial transformation of the nitrogen contained. If the experiments had extended through the period of most active decomposition which occurs usually in late May and June, no doubt entirely different results would have been obtained. It suggests the desirability of more investigation along this line.

Hutchinson and Milligan (2) studied the decomposition of a legume crop in the green condition, and after drying for 24 and 48 hours, in connection with the rate of ammonia, nitrite and nitrate production. This work is pertinent to the data presented by the authors of the present article. The legume used by Hutchinson and Milligan was *Crotalaria juncea* and the samples were cut from plantings four weeks old. This young succulent growth possessed a moisture content of 452.1 per cent on the water-free basis, or 81.8 per cent of the green material. The results in table 1 are taken from their bulletin.

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[1] The work reported in this publication was completed in 1916 but publication was delayed on account of the war. An article published by J. W. White (Soil acidity as influenced by green manure. *In* Jour. Agr. Res , 1918, v. 13, p. 171–197) supports the data herein reported as regards the rate of decomposition of green and air-dried materials.

By calculating from the authors' data, it will be found that the total inorganic nitrogen transformed was 68.1 per cent, 57.3 per cent, and 47.1 per cent, for the crop buried immediately, that buried after drying for 24 hours, and that buried after drying for 48 hours, respectively. When, however, the nitrate alone is considered the differences are much greater in favor of the "buried immediately" material. The production of ammonia was somewhat greater with the treatments which had partially dried. The presence of nitrite for the first seven days is of considerable importance to note, as the amount is sufficiently high to be injurious to most vegetation.

TABLE 1

Effect of drying green crop before burial

TREATMENT[*]	NITROGEN DETERMINED	NITROGEN PER 100 GM SOIL						
		1 week	2 weeks	3 weeks	4 weeks	6 weeks	8 weeks	12 weeks
		mgm.	mgm.	mgm.	mgm.	mgm	mgm.	mgm.
Buried immediately	Ammonia	6 9	2 5	1 0	2 0	1 0	2 0	2 0
	Nitrite	1 2	0 01	nil	nil	0 01	0 01	0 02
	Nitrate	3 1	6 9	8 4	8 9	9.4	9 8	8 7
							68 0%[†]	
Buried after drying for 24 hours	Ammonia	6 5	3 5	2 5	3 5	3.5	3 5	2 0
	Nitrite	1 1	0 02	0 02	nil	0 02	0 03	0.02
	Nitrate	4 1	5 1	6 0	6 9	6 9	6 9	6 5
							45 3%	
Buried after drying 48 hours	Ammonia	5 4	3 5	4 0	4 0	2 5	3 0	2 5
	Nitrite	0 05	0 02	0 03	nil	0 02	0 03	0 02
	Nitrate	3.8	5.1	5.3	5 3	5 4	6 1	5.8
							39 1%	

[*] 12.8 mgm. of nitrogen were added in green material per 100 gm. of soil.

[†] Per cent nitrified of organic nitrogen added.

Another point of interest that appears throughout the whole work of these investigators is that the maximum nitrate production was found to occur at the eighth week from the time of burial.

EXPERIMENTAL METHODS

The experiment reported in this paper was planned with the object of making a comparative study of the rate of decomposition of red clover tops, turned under green and cured by drying, all other conditions and factors being the same in both cases. The rate of decomposition was studied by experiments conducted in both the laboratory and the greenhouse. The main points considered were the transformation of the nitrogenous compounds, loss of carbon, and the effect upon growing crops planted at various periods after the beginning of the experiment.

General plan

The experiment was conducted in parallel in the greenhouse and in the laboratory. Twelve 1-gallon earthenware jars were prepared for the greenhouse experiment; four containing the soil alone, four a mixture of the soil and green clover tops, and four a mixture of the soil and cured clover tops. Two jars of each treatment were selected to draw samples from, for the analytical work, and the other two were reserved for growing oats. In order to make the chemical determinations more accurate, it was necessary to make a rather heavy application of organic matter. Consequently, the clover was applied at the rate of 50 tons of green manure per two million pounds of soil. In the laboratory experiment ½-pint jelly glasses, covered with tin lids in which a small hole had been punched, were used for containers. Sixty glasses were prepared, twenty for each of the above mentioned treatments. The applications of organic matter were approximately twice as much as in the 1-gallon jars.

The additions of green and cured clovers were in equivalent amounts; that is, the loss of moisture in curing was determined and the applications were made so that every jar received the same amount of organic matter. At the time the clover was mixed with the soil, the cured-clover-soil mixture was moistened with sufficient distilled water to bring it to the same moisture content as the green, thus both treatments were uniform with respect to moisture content.

Materials used and their preparation

The soil used was a heavy phase of brown silt loam. The clover was of a late fall growth, and represented both young and old plants, some which were large and woody with dried-up blossom heads. The original sample was thoroughly mixed and divided into two portions of 1500 gm. each. One portion was spread out thinly on a sheet of cheesecloth and dried for three days in the breeze from electric fans. In the three days of curing the weight of the clover decreased to 441 gm. The other portion was stored in shallow trays in a refrigerator at a temperature a few degrees above the freezing point. The green clover weighed 1504 gm. when removed from the refrigerator. Both lots were cut into pieces of approximately ½ inch in length.

Details of greenhouse experiment

Three lots of soil weighing 16 kgm. each were placed in piles on the greenhouse bench. To one lot was added 720 gm. of the chopped green-clover tops; to the second 211.6 gm. of chopped, cured-clover tops and 508.4 gm. of distilled water. The added materials were thoroughly mixed throughout the whole soil mass. To the third lot of soil no addition was made, but it was subjected to the same mechanical treatment as in the other cases. Four 1-gallon jars were filled with each mixture and four with soil alone, each jar receiving a total of 4000 gm.

The soil used was obtained directly from the field and when collected, contained an optimum moisture content, consequently no water was added except in cases where a deficiency resulted due to handling or where rapid evaporation from the green clover occurred. The total weights of the jars were recorded and they were periodically brought up to the original weight by the addition of nitrogen-free distilled water. The original moisture content was lowered somewhat as decomposition progressed, because of the larger amount of available moisture resulting from decreased capacity for absorption. The soil in these jars was cultivated and thoroughly stirred after each sampling. On the third day after starting the experiment one jar of each treatment was planted with 16 selected seeds of Silver Plume oats, and on the twenty-first day, another jar of each treatment was planted with 20 selected seeds of the same variety.

Details of laboratory experiments

In each of the sixty jelly glasses was placed 120 gm. of the same lot of soil as that used in the greenhouse experiment. The first twenty received in addition 10 gm. each of green-clover tops, which had been chopped as previously described. To the next twenty glasses were added in a similar manner 2.93 gm. of cured-clover tops and 7.1 gm. of distilled water. The remaining twenty glasses served as checks. The glasses were all weighed and whenever necessary enough water was added to restore them to their original weight. They were cultivated after each watering. These treatments were incubated at room temperature in the laboratory. In order to test the effect of a limited supply of oxygen on the decomposition, four shaker bottles were added to the experiment. In the first two were placed 120 gm. of soil and 10 gm. of green-clover tops, while the other two received 120 gm. of soil, 2.93 gm. of cured-clover tops and 7.1 gm. of distilled water. These bottles were stoppered tightly and incubated at room temperature for seven months. The details of the experimental plan are arranged in table 2.

Sampling and analytical methods

In sampling, the whole of the contents of the jars in the greenhouse was stirred with a large spatula and then an equal weight of soil was taken from each of two jars of the same treatment. The soil was then mixed together, making a composite sample of the same treatment from two jars. In the laboratory the total contents of two jelly glasses from each treatment were mixed in the same manner as the samples from the jars. Samples were taken in this manner to avoid, as much as possible, individual differences among the containers.

Moisture determinations were made by drying in the electric oven at 110°C. in the usual manner. Ammonia nitrogen was determined in duplicate on all of the samples by aeration with magnesium oxide at room temperature. This method was first developed in this laboratory. Since this work was completed, results showing the value of magnesium oxide in aeration methods for ammonia determinations in soils have been published from the Ohio Experiment Station and therefore need not be repeated here.

TABLE 2

Details of experimental plan

TREATMENT	SOIL	CLOVER	WATER	MOISTURE PRESENT	CLOVER ADDED	NITROGEN ADDED	TOTAL NITROGEN PRESENT
Materials used in four jars							
	gm	*gm.*	*gm.*	*per cent*	*p p m*	*p p m*	*p p.m.*
Greenhouse:							
Soil and green clover.	16,000	720 0	0	33 00	14,163	497 1	4,084
Soil and cured clover	16,000	211 6	708 4	33 00	14,163	437 8	4,036
Soil alone .	16,000	0	308 0	29 87	0	0	3,522
Material used per glass							
Laboratory:							
Soil and green clover.	120	10 0	0	33 25	25,215	885 6	4,331
Soil and cured clover .	120	2 93	7 1	33 25	25,662	792 0	4,236
Soil alone	120	0	4 0	30 38	0	0	3,535

For the nitrate determinations

The dried soil was put into 400-cc. shaker bottles and 300 cc. of hydrochloric acid (5 cc. of concentrated acid in a liter) were added to each bottle. The bottles were then shaken 20 minutes, and then allowed to settle over night. Two hundred cubic centimeters of the supernatant liquid were removed and placed in Kjeldahl flasks. Six cubic centimeters of potassium hydroxide solution (600 gm. per liter) were added and the ammonia expelled by boiling almost to dryness. One gram of Devarda's alloy was added to each flask together with 250 cc. of water. The distillation was carried out in the usual manner. Rosolic acid was used as the indicator in titrating.

The total nitrogen and total carbon were determined on the air-dried samples by the Kjeldahl and sodium peroxide fusion methods, respectively. Slight differences were found between the nitrogen content of the green and cured clover tops. The differences were not, however, sufficient to affect the determinations. The slightly lower nitrogen content of the cured clover is unaccountable, but similar results have been observed by other investigators.

EXPERIMENTAL RESULTS

Table 3 shows the ammonia and nitrate content of the various treatments at different periods in the progress of the greenhouse experiment. The figures show that the decomposition of both kinds of clover was fairly rapid. The maximum ammonia content was found at the beginning of the experiment. There was a gradual decrease to about the normal production for the brown silt loam soil. There was not the slightest indication of a very rapid accumulation of ammonia in the early days of the experiment such as has been found repeatedly in this laboratory with green alfalfa or dried blood. The difference in ammonia production between fresh and cured clover is not such as to indicate anything of value regarding the rate of decomposition of the two materials. This, of course, was to be expected in a soil of this type. Ammonification is not an index as to the rate of decomposition. The production of nitrate from the fresh clover proceeded rapidly from the beginning, and continued well in the lead for two months over the production from the cured clover. On the eighty-ninth day the nitrate content of the two treatments was practically the same, and on this date also both treatments showed a maximum increase in nitrate over the soil alone. The proportion of the original nitrogen added transformed into nitrate for the fresh clover was 31.34 per cent, and for the cured, 34.35 per cent.

It is to be noted that the important differences occurred during the first eight weeks. This more rapid decomposition on the part of the green material is an important consideration, particularly in connection with a crop such as corn, which is planted after the plowing under of a green manure about the first of May. If differences of this kind occur between fresh and cured clover, such as that used in the experiment, it is reasonable to assume that much greater differences would occur with clover which had been subjected to longer curing. Curing appeared to have a retarding effect in the early stages of decomposition, but the effect was not noticeable after two months.

The results obtained in the laboratory are shown in table 4. The largest content of ammonia was found in the green-clover treatment at 10 days. Because of the larger amount of material used in the laboratory experiment, a much greater accumulation of ammonia was to be expected, especially with the green clover. The amount found, however, is only about 9 per cent of the nitrogen applied in the case of green clover and less than 3 per cent in the case of cured clover at 10 days, when the maxima occurred. At 10 days the green clover was still higher than the cured, but from that time on there was little difference between the two treatments. The results of the nitrate determinations are much more striking than those of the ammonia. The green clover produced nitrate very rapidly with a maximum transformation of 35.8 per cent of nitrogen added after 43 days. The cured clover produced nitrate more slowly; only 15.7 per cent of the nitrogen applied was found in the form of nitrate at the end of 43 days.

The maximum nitrate accumulation for the cured clover occurred at 152 days when 24 per cent was nitrified. The largest actual amount of nitrate found was 347.1 parts per million, and whenever the nitrate approached this amount, a drop occurred followed by another approach to the maximum. This was thought to be due to a sufficient concentration of the nitrate to inhibit biological action, but upon calculation it was found that this could not be a factor, and it appears that an equilibrium may occur between protein formation and nitrification when the maximum is reached. This equilibrium may be broken by protein formation exceeding nitrification, and vice versa. Total nitrogen determinations gave no evidence that denitrification was taking place. The effect of curing on ammonification was of no importance, but a very marked effect was observed on nitrate production. This effect was much greater in the laboratory experiment than in the greenhouse experiment. Although almost the same maximum percentage of nitrate production was found from both the fresh and the cured clover, the attainment of this max-

TABLE 3

Ammonia and nitrate nitrogen content of soil treatments at different periods in greenhouse experiment

TREATMENT	NITROGEN AS PARTS PER MILLION OF WATER-FREE SOIL								
	0 days	10 days	18 days	30 days	43 days	59 days	89 days	152 days	213 days
Ammonia									
	ppm	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
Soil and green clover	23 9*	21 7	14 0	11 3	15 6	13 1	4 6	13 5	7 5
Soil and cured clover	27 8	17 6	7 5	3 9	21 9	9 6	4 9	13 8	8 6
Soil alone	9 4	9 6	6 3*	4 3	8 6	6.3	3 1	—	5 6
Nitrate									
Soil and green clover	10 2	80 4	101 1	112 3	102 5	122 3	197 8	163 4	203 7
Soil and cured clover	10 6	56 1	85 4	83 1	93 8	103 9	192 4	84 4*	178.4
Soil alone	9 4	36 6	32 8	24 8	24 9	24 6	42 0	43 5	51 6

* Single determination; all the others are averages of duplicates.

imum was delayed 109 days by the curing in the laboratory experiment. Whether the difference between the laboratory and the greenhouse experiments was due entirely to the different rates of application of the clover or to some other influences cannot be told from the data at hand. It is evident that both methods are sufficient to indicate the direction the decomposition takes, and data obtained by these methods are perhaps as reliable as the results obtained under field conditions. The data do not indicate that the kind of decomposition in the case of the fresh and cured material was essentially different. The per cent of nitrogen changed to nitrate was almost the same in both laboratory and greenhouse methods. This per cent was not influenced by the curing or by the rate of application.

TABLE 4

Ammonia and nitrate nitrogen content of soil treatments at different periods in laboratory experiment

TREATMENT	NITROGEN AS PARTS PER MILLION OF WATER-FREE SOIL								
	0 days	10 days	18 days	30 days	43 days	59 days	89 days	152 days	213 days
					Ammonia				
	p p m.	*p p m*	*p p m.*	*p p m*	*p p m*	*p p m*	*p p m.*	*p p m.*	*p p.m.*
Soil and green clover .	48.9	89.6	34.8	13.5	21.5	14.6	3.2	19.2	5.4
Soil and cured clover	36.1	36.7	20.0	14.0	20.0	17.8	8.6	19.1	8.4
Soil alone	9.6	8.1	2.7	8.1	7.0	8.7	5.8	7.5	3.7
					Nitrate				
Soil and green clover. . .	10.0	95.9	206.0	269.6	347.1	338.9	300.5	289.6	326.7
Soil and cured clover. . .	10.0	71.2	114.4	130.8	154.7	197.4	266.8	332.9	309.0
Soil alone	9.4	18.0*	26.1	24.5	30.2	49.2		63.4	66.0

* Single determinations; all the others are averages of duplicates.

Decomposition in presence of a limited supply of oxygen

The senior author had occasion to observe a very acid decomposition of green vetch which appeared to be due to an excess of moisture. In order to test the possibility of clover producing the same kind of fermentation, both green and cured clover were subjected to conditions of a limited supply of oxygen by incubation in stoppered shaker bottles as described above. The decomposition under these conditions was found to be widely different. In table 5 are included the ammonia and nitrate results.

The high accumulation of ammonia with the green clover is sufficient under normal conditions to stop nitrate production in this soil, but not sufficient to stop nitrite formation. The cured clover produced a large amount of nitrate and actually consumed in so doing about 66 per cent of the available oxygen. The green-clover treatment possessed the odor typical of silage and was extremely acid. It should be observed that this is not a case of denitrification, but a case of an acid fermentation in which the initial stages of ammonification were not inhibited. It suggested that an acid fermentation may result under field conditions when the green decomposing material is in any way sealed up by water or by a blanket of carbon dioxide in the soil. Further work will be conducted along these lines, as it appears that this condition is not infrequently met with in wet seasons, and is a probable cause of injury to crops planted after plowing under heavy growths of green manures. The stems and leaves were in a fair state of preservation after 7 months in these treatments, while under more open conditions only small portions of the woody stems were found.

TABLE 5

Ammonia and nitrate nitrogen produced by green and cured clover with a limited oxygen supply

Duration 213 days

TREATMENT	NITROGEN	
	Ammonia	Nitrate
	p p m	*p p.m.*
Soil and green clover	224 9	6 0
Soil and cured clover	106 6	97 2

Loss of carbon

The loss of carbon during 152 days of the experiment is reported in table 6. While there is a relatively large error in the total carbon determinations, nevertheless these results show distinct decreases in the carbon, and in all cases the green clover lost considerably more than the cured.

TABLE 6

Loss of carbon during 152 days of decomposition

TREATMENT	CARBON LOST
	p p m.
Greenhouse:	
Soil and green clover	2456
Soil and cured clover	2214
Laboratory:	
Soil and green clover	8218
Soil and cured clover	5857

Carbon and nitrogen relationships

The ratio of total carbon to total nitrogen, and the ratio of carbon lost to nitrate formed, are given in table 7 in order to show the rapid narrowing that occurred in a relatively short time, and to further point out the fact that the carbon-nitrogen ratio of the green-clover treatment narrowed faster than that of the cured-clover treatment. The ratio loss of carbon to nitrate formed was much greater with the green-clover treatment than with the cured-clover treatment. These ratios represent quite accurately the relative rates of decomposition of the two materials and the relative rates as they occurred in the two experiments. The carbon-nitrogen ratio indicated that the green clover had reached a more advanced stage of decomposition than the cured clover.

TABLE 7

Carbon and nitrogen relationships

TREATMENT	RATIO OF CARBON TO NITROGEN		RATIO OF CARBON LOST TO NITRATE FORMED
	0 days	152 days	C to N
	C to N	C to N	
Greenhouse:			
Soil and green clover.	12 4-1	11 7-1	12 3-1
Soil and cured clover .	12 4-1	11 8-1	11 5-1
Laboratory:			
Soil and green clover	12 8-1	10 9-1	23 6-1
Soil and cured clover .	12 6-1	11 1-1	17 6-1

Effect of decomposition of green and cured clover on oats

One jar of each treatment was planted with Silver Plume oats 3 days after the experiment was started and 21 days after starting, a second planting was made in three other jars. The oats from the first planting were up after 4 days. Injury in the germination was noticeable where the organic matter was present and it was greater with the green clover. Only 6 seeds out of 16 germinated at all and these very poorly where the green clover had been applied, while 10 germinated where the cured clover was applied and 16 in the soil check. For about two weeks the plants in the green-clover treatment were much behind those in the other treatments, but gradually the differences disappeared. The oats were germinated at a time when the temporary ammonia accumulation from the green clover might account for the injuries observed. No injuries were observed with the oats planted 21 days after the applications were made. In both cases at the close of the experiment the green and cured treatments were ahead of the checks.

DISCUSSION

Hypothetical reasons for differences in rate of decomposition between green and cured clover

In considering the greater initial rate of decomposition exhibited by the green clover as found by all methods used to study the decomposition, it did not appear that there were any indications of purely chemical changes having occurred in the clover during dehydration. Enzymic action as a factor in affecting the cell contents is not an impossibility, but the temperature at which the green clover was held during the dehydration of the lot with which it was compared, would exclude the possibility of much change in it, while the dehydration process with the other lot of clover would require the action of the synthetic enzymic processes to retard its rate of decomposition by stabilizing the nitrogen compounds.

The explanation that seems to fit the results found is based on a physical hypothesis. The loss of water is of course accompanied with an increase in the concentration of salts in the cells. This brings about a hardening of the cell contents, and the whole material becomes more horny and shriveled. The diffusion of nitrogenous substances is stopped until hydration has been reestablished. The colloidal nature of the cell contents causes them to become hydrated slowly, after a previous dehydration. Apparently the time required to bring about the hydration in a normal soil is sufficiently long for the ammonifying bacteria to gain headway on the green material, and consequently to effect a much greater rate of decomposition than those acting on the dehydrated material.

CONCLUSIONS

1. The comparative rate of decomposition of green and of cured red clover tops was studied in soil. Curing retarded the rate of decomposition as measured by ammonification, nitrification and loss of carbon in both laboratory and greenhouse experiments. The green clover produced nitrate very rapidly, with a maximum transformation in the laboratory experiment at 43 days of 35.8 per cent, while with the cured clover at the same period only 15.7 per cent of the nitrogen had been transformed. In the greenhouse experiment the green clover was well in the lead in nitrate production during the first two months.

2. Green and cured red clover underwent the same kind of decomposition under aerobic conditions.

3. With the oxygen supply limited, the types of decomposition of green and cured red clover were vastly different.

4. There was no measurable loss or gain of nitrogen during the experiment.

5. The loss of carbon, and the change in the carbon-nitrogen ratios agreed with the other determinations in showing a difference in rate of decomposition between the green and the cured clover, but did not indicate a difference in kind.

6. The change which dehydration (curing) brought about in the rate of the initial decomposition appears to be of a physical nature only. An explanatory hypothesis is that dehydration resulted in a hardening and shriveling of the tissues which interferes with the reentrance of water and consequently delays the decomposition because the bacteria must await the softening of the tissues before they are able to start their work, while with the green no such delay occurs, as the cells are already hydrated.

7. Planting oats 3 days after treating the soil with green and cured clover resulted in serious injury which delayed growth. It was greater with the green clover.

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CONCERNING THE EFFECT OF HEAT ON THE REACTION BETWEEN LIME-WATER AND ACID SOILS

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Lime-water (calcium hydroxide) added to so-called "acid soils" as an index of the lime requirement, has been generally employed by analysts since Veitch (5) outlined his method in 1902. Many workers throughout the country consider the method superior to any other as an indication of the total lime requirement of a soil and advise treatment accordingly. In scientific investigations of soil problems pertaining to soil acidity and general fertility, the method is often used to show various changes that have occurred. Whenever a new method for soil acidity or lime requirement has been suggested the Veitch lime-water method is usually taken as a means of comparison. A similarity of results is often considered desirable. In view of these conditions it is important that we have as complete a knowledge as possible of all factors that would influence the results obtained by the method in question.

During the course of an investigation pertaining to the acid condition of Oregon soils it was observed that the lime requirement of an acid soil, as indicated by the Veitch (5) lime-water method, was greater after treatment with limestone than before treatment. Since the first determination was made on a hot-water bath while the later one was carried out on a sand bath heated by a coil of steam pipes, it was thought that the degree of heat or manner of evaporation might be an important controlling factor and account for the inconsistent results. Accordingly, a study was made to ascertain the various conditions that affected the lime requirement as shown by results of the lime-water method.

Five acid soils, widely different both physically and chemically, were used in the investigation. A physical classification as shown by a mechanical analysis is as follows:

No. 11076	Silty loam
No. 11077	Gravelly loam
No. 11078	Williamette silty loam
No. 11079	Clay loam
No. 11080	Medium sandy loam

The soil samples were air-dry, and, except for a limited amount of grinding with a rubber-tipped pestle, were used as obtained from the field.

In all determinations of the lime requirement the procedure was followed as recommended by Veitch (5). The quantity of soil used each time was 10 gm. Water was first added to the soil to make the final amount 65 cc. after the standard lime-water was added, except for those soils having an abnormally high requirement. Evaporations were then carried on, as will be described later. After drying, the soils were taken up with distilled water and transferred to 250-cc. centrifuge bottles, 75 cc. being the total amount of water used. The bottles were shaken occasionally during 3 hours of the afternoon and allowed to stand over-night. Instead of decanting through filter paper, the bottles were placed into a centrifuge and whirled at the rate of about 2400 revolutions per minute until a clear solution was obtained. After the addition of phenolphthalein indicator, 50 cc. of this solution was then boiled until the appearance of the pink color, or, in case no color developed, to about 5 cc. Care was taken to maintain the above conditions in order that the ratio of soil to water would be constant in all determinations and to control other influencing factors. Control determinations were run to ascertain any alkalinity that might develop from the use of the glass apparatus. The 200 cc. of the distilled water evaporated down to 5 cc. in the Pyrex beakers used gave no trace of alkalinity to phenolphthalein indicator.

TABLE 1

The effects of heating on the lime requirements of a soil by the Veitch method

AMOUNT TAKEN	METHOD OF EVAPORATION	TEMPERATURE	TIME FOR EVAPORATION	LIME REQUIREMENT ($CaCO_3$ FOR 2,000,000 POUNDS OF SOIL)
gm		°C.		*lbs.*
10	On steam bath	100	1 0 hr	2600
10	On steam bath	90	1 5 hr.	2150
10	On steam bath	70	2 5 hr.	1300
10	On steam bath	100	1 0 hr.,—left 10 min after dehydration	3200
10	On steam bath	100	1 0 hr ,—left 20 min after dehydration	3400
10	In vacuum oven	75	About 20 hr.	3900
10	In vacuum oven	60	About 8 hr.	1700
10	In vacuum oven	60	About 10 hr.	2150
10	On hot plate	Low heat	2 hr.	4300
10	On sand bath	110	About 8 hr.	4600

Soil 11078 was selected for a detailed study pertaining to the effect of heat during the process of determining the lime requirement by the Veitch method. This soil type gave a distinct acid reaction to litmus paper and had a lime requirement of 1 to 3 tons per 2,000,000 pounds of soil, depending upon the laboratory method used for the determination. It does not, however, respond to lime treatment, as indicated by crop production. Evaporations of the treated soil were conducted on a hot-water bath (called steam bath by

Veitch) at different temperatures controlled by gas burners; on a sand bath heated by steam coil pipes; on a hot plate at "low heat" connections; and in a vacuum oven at various degrees of temperature controlled by gas burners. Table 1 gives the lime requirement as indicated by the conditions under observation. The results are reported as pounds of calcium carbonate per 2,000,000 pounds of soil.

These results show a wide variation in the lime requirement of an acid soil, depending upon the manner of evaporation after treatment with standard calcium hydroxide. When the evaporation was conducted on the steam bath highest results were obtained at a temperature of 100°C. Any decrease in temperature showed a corresponding decrease in the lime requirement. At a temperature of 70° the lime requirement was 1300 pounds, while at 100° it was 2600 pounds, or 100 per cent higher. Furthermore, if the treated soil was allowed to remain on the steam bath 20 minutes after evaporation to dryness was completed, the lime requirement was 3400 pounds, or almost three times as much as was indicated when the treated soil was evaporated at 70° and removed immediately upon drying. The results just cited emphasize the influence of heat on lime requirement. Also when evaporation of the treated soils were conducted in the vacuum oven, the temperature appeared to be a controlling factor. At 60° in vacuo, when it required between six and seven hours to complete evaporation, the lime requirement was 1700 pounds. Any increase either in temperature or time permitted to remain in the oven after complete dehydration of treated soil further increased the lime requirement. These results show that heating at a lower temperature than 100°, namely at 60° or 75°, after evaporation has been completed, also influences the results similarly to those obtained on the steam bath where the soil was allowed to remain a short time after drying. Attention is called to the high lime requirement indicated when evaporation was conducted on the sand bath. The temperature of the sand in the sand bath varied from 70°, on the surface, to 110°, 1.5 inches below the surface proximate to the steam coil. As shown in the table it required about 8 hours to complete evaporation. This is due, probably, to the small amount of surface of the dish contiguous to the source of heat. Care was n taken to remove dishes on the sand bath immediately after dehydration and the dried soil may have been heated 15 minutes or more. If continued heating of the dried soil increases the amount of calcium hydroxide taken up, these higher results would be expected.

No difficulty was experienced in obtaining consistent results for each determination under the conditions described. Duplicate determinations gave very close results, provided the source of heat, temperature and digestion of the treated and dried soil were identical.

The other soils varying particularly in their physical properties and degree of acidity gave similar results to those observed above. In all cases the soils were removed immediately after dehydration in order to avoid excess heating of the dried soil. Table 2 gives the lime requirement of these soils when evaporated under the different conditions indicated.

All of the soil types showed a wide variation in the lime requirement, depending upon the manner in which the treated soil was evaporated. In soil 11079 we note a difference of 5500 pounds between two methods of evaporation, while in soil 11080 there is a difference of almost 10,000 pounds. Differences of this magnitude show that the lime-water method does not give an accurate index of the actual lime requirement of an acid soil.

Examination of the results thus far reported indicate that the higher the temperature at which the dry portion of the soil is subjected during the time when the undried portion is dehydrating, the higher will be the lime requirement. The reaction that occurs during the evaporation of the treated soil is apparently influenced by the temperature at which the dried portion of the soil is subjected during the process of evaporation. It is improbable that the reaction takes place in the undried portion of the soil during evaporation, although abnormal results were obtained in vacuo at 60°, which is a very low temperature compared with the actual temperature of the mixture of soil and water evaporating on a hot-water bath at 100°. It has been observed that some soils in Oregon have a higher lime requirement, as indicated by our laboratory methods, in late summer than in the spring. The fact that heating the soil even at low temperatures, as in vacuo at 60°, apparently affects the amount of lime-water taken up, may explain the cause of seasonal changes in the lime requirement.

TABLE 2

The effect of heating by various means on the lime requirement of soils

SOIL NUMBER	AMOUNT TAKEN	METHOD OF EVAPORATION	LIME REQUIREMENT ($CaCO_3$ FOR 2,000,000 POUNDS OF SOIL)
	gm.		*lbs.*
11076	10 0	Steam bath	3,200
11076	10 0	Sand bath	3,900
11076	10 0	Hot plate	5,400
11077	10 0	Steam bath	1,500
11077	10 0	Sand bath	1,700
11077	10 0	Hot plate	2,400
11079	10 0	Steam bath	10,000
11079	10 0	Sand bath	12,000
11079	10 0	Hot plate	15,500
11080	10 0	Steam bath	20,800
11080	10 0	Sand bath	24,200
11080	10 0	Hot plate	30,000

It is well known that soils undergo chemical changes when heated even at low temperatures. One of the important reactions is the increase in the water-soluble constituents. Under the influence of heat both mineral and organic constituents of the soil are rendered more soluble. Furthermore, it has been found that the soluble material increases at higher temperatures In the presence of moisture or water the organic material is still further transformed into the soluble state. Under these conditions, therefore, it is probable that the reaction between the soluble salts and calcium hydroxide would be increased. Also, more calcium hydroxide would probably be absorbed by a soil made deficient in bases by increased solubility of its mineral and organic constituents. Consequently, under these circumstances a soil would show a higher lime requirement by the lime-water method, depending upon chemical changes caused by heating.

Other investigators have observed several factors that affect the lime requirement of the soil as judged by the Veitch method. Brown and Johnson (1) found that grinding a soil decreased its apparent acidity, especially with coarse sandy soils. They stated that grinding to a fine powder facilitated solution and hydrolyzation of minerals contained in the soil, giving increased basicity. This was in accordance with the view held by the Rogers Brothers (4) who showed that many of the common minerals, particularly the zeolites and feldspars, when reduced to fine powder and treated with water, gave an alkaline reaction with phenolphthalein indicator. Conner (2) found that keeping soils at different moisture contents alters their acidity. Noyes (3) reported that a soil containing residual limestone gave an acid reaction to the Hopkins method, but when treated with water and evaporated on a steam bath it gave an alkaline reaction. Veitch (6) in a critical study of his lime-water method, noticed that two of the soils studied were more acid when the treated soil was dried in the open air than when dried on the steam bath. He stated that he "did not have time to make further examination of these cases." It is probable that the soils referred to by Veitch contained residual limestone similar to those reported by Noyes, since they were more alkaline after being heated on a bath. Soils 11076 and 11078 gave a very alkaline reaction when several small particles of hard limerock were added and, after treatment with water, evaporated on the steam bath. All of these observations emphasize the fact that the chemical composition and physical properties of a soil have a specific effect on the amount of calcium hydroxide necessary to satisfy the apparent acidity when artifically heated.

In a critical study of his lime-water method Veitch (6) observed that when the treated and dried soil was allowed to remain in contact with water for 48 hours or more, it developed the pink color of phenolphthalein with less lime-water than if allowed to remain in contact 16 hours or less. The wide variation in the chemical composition and physical properties of the soils used in this study suggested the possibility that similar results might not be obtained. Consequently, it was thought that a study of this phase of the subject was worthy of consideration. The procedure was followed as outlined for the previous experiments but instead of shaking the treated and dried soil with water for 16 hours it was also allowed to remain in contact for 40 hours.

Table 3 gives the amount of calcium carbonate equivalent to the lime requirement of the soil allowed to remain in contact with water as indicated.

This table presents some very interesting results. All the soils showed a reduced lime requirement when permitted to remain in contact with water for longer periods of time. Furthermore, each showed that a different reaction had occurred. During the increased time for digestion soil 11076 increased in basicity so that the lime requirement was reduced over 50 per cent; soil 11080, a very acid type, was reduced only about 20 per cent; soil 11079, also a very acid type, was reduced almost 70 per cent; soil 11077 developed basicity almost to actual alkalinity after 40 hours and when the natural soil, untreated with lime-water, was allowed to remain in contact with distilled, carbon-dioxide-free water another 24 hours or a total of 64 hours it gave an alkaline reaction. It may be mentioned at this time that this soil gives a distinctly acid reaction to all other laboratory methods, both qualitative and quantitative, for the determination of soil acidity. Veitch explains that his development of basicity may be due to the slower solubility and hydrolyzation of neutral compounds that were formed by the lime-water added, or to the hydrolization of neutral compounds already in the soil. If the peculiar action of soil 11077 can be explained on this basis it is evident that the reaction between the soil and the water constituting the normal moisture content of the soil is different from the reaction between the soil and a large amount of water added, for in no case has the soil ever shown an alkaline reaction as indicated by testing a water extract of the moist soil taken from the field.

TABLE 3

Lime requirement of soils allowed to remain in contact with water different lengths of time

SOIL NUMBER	METHOD OF EVAPORATION	LENGTH OF TIME SHAKEN	LIME REQUIREMENT ($CaCO_3$ FOR 2,000,000 POUNDS OF SOIL)
		hours	*lbs*
11076	Steam bath	16	3,200
11076	Steam bath	40	1,500
11077	Steam bath	16	1,500
11077	Steam bath	40	100
11077	Steam bath	64	Alkaline
11079	Steam bath	16	10,000
11079	Steam bath	40	3,400
11079	Hot plate	16	15,500
11079	Hot plate	40	5,200
11080	Steam bath	16	20,800
11080	Steam bath	40	18,100

CONCLUSION

Results obtained by a study of the Veitch lime-water method for soil acidity warrant the following conclusions:

1. The lime requirement of a soil as shown by the lime-water method indicates a reaction that takes place under the conditions performed, and the results will be affected by any variation of the following factors:

   a. The physical or chemical properties of the soil.
   b. Probably by the ratio of amount of soil to quantity of solutions to be evaporated.
   c. The temperature at which evaporation is made.
   d. Continued heating after the soil has been dehydrated.
   e. The length of time during which treated and dried soil remains in contact with water.
   f. The source of heat, namely, steam bath, sand bath, hot plate, etc.

2. Results will be comparative when the lime-water method is used if the determinations are conducted in exactly the same manner.

3. The data presented above emphasize the necessity of controlling all influencing factors if the lime-water method is used to observe changes in a soil by comparison of results obtained at different times.

REFERENCE

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THE MOISTURE EQUIVALENT IN RELATION TO THE MECHANICAL ANALYSIS OF SOILS

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Mechanical analysis has been used as a basis for the classification of soils for several years. This method of classification is not altogether satisfactory in many respects, but it is the only method so far devised which gives comparable results by routine methods.

Moisture equivalent is defined as the maximum percentage of moisture which a soil can retain in opposition to a centrifugal force equal to 1000 times the force of gravity. The objects of the present investigation were to determine whether or not the moisture equivalent could be used as a basis to assist in the classification of soils, and what relation, if any, existed between the moisture equivalent and the mechanical analysis.

The moisture-equivalent determinations were made according to the method of Briggs and McLane (1), on samples of soil received by the Division of Physical Investigations from the Division of Soil Survey for mechanical analysis.

The apparatus for the determination consists of a metal cylinder attached to the shaft of a vertically mounted electric motor. The motor is provided with a governor which keeps the speed within the desired limits, a speed of 2440 revolutions per minute being necessary for a centrifugal force equal to 1000 times the force of gravity. The cylinder holds 16 soil boxes which have wire gauze bottoms permitting the escape of the excess moisture. The gauze bottoms are covered with filter paper to prevent the loss of soil particles, and then the soil is placed in the boxes, a sufficient amount of soil being taken to give a packed layer approximately 1 cm. thick. Duplicate determinations are made, the duplicate samples being placed opposite to one another in the cylinder. The samples, after being placed in the boxes, are thoroughly moistened and allowed to stand in a saturated atmosphere for 24 hours, a little more water is added, and they are placed in the cylinder of the machine. The machine is run for forty minutes at the desired speed, the samples are removed, and a moisture determination made upon each one. The percentage of moisture, based upon the weight of the dry soil. is the moisture equivalent. A total of 172 duplicate determinations were made on samples from counties in various states as follows:

La Salle, La	26
Horry, S C	19
Morgan, Ala	20
Waupaca, Wis	17
Bertie, N C	12
Brawley, Cal	9
El Centro, Cal	9
Newberry, S. C	2
Willits, Cal	3
Bannock, Ida	1
Kent, Del	1
Fort Laramie, Wyo	2
Lubbock, Tex	5
Adair, Iowa	4
Cheyenne, Nebr	23
Braxton and Clay, W Va	2
Chenango, N Y	5
Aroostook, Me	17
Total	172

In order to determine whether or not the moisture equivalent could be used as a basis of classification, the determinations were divided into classes as determined by their mechanical analysis The results of this grouping are shown in table 1 The mean value of the determinations in each class shows a gradual increase, on the whole, from class to class, with the notable exception of silt loam, but the minimum and maximum determinations show considerable variation and overlapping among the various classes. From the results shown in this table it is readily seen to be impossible to base a classification upon the moisture equivalent which would be in agreement with the present classification. However, it shows the value which the moisture equivalent may have in the interpretation of the mechanical analysis.

TABLE 1

Moisture equivalent of soils grouped according to soil classification

NUMBER OF SAMPLES	DESCRIPTION	MOISTURE EQUIVALENT		
		Minimum	Maximum	Mean
2	Coarse sand	2 15	7 80	4 98
3	Sand	3 00	6 55	4 77
7	Fine sand	6 65	11 35	8 50
3	Very fine sand	9 70	12 85	10 75
9	Sandy loam	8 95	14 80	12 88
47	Fine sandy loam	9 75	25 25	16 56
9	Sandy clay	15 55	34 25	22 76
24	Loam	15 40	29 15	22 29
19	Silt loam	18 25	41 25	26 75
20	Clay loam	16 35	32 90	22 29
12	Silty clay loam	19 80	29 90	26 90
17	Clay	20 85	40 20	29 25

Since there was no correlation between the classification of soil and the moisture equivalent, there remained to be determined whether any relation existed between the moisture equivalent and the mechanical analysis. Briggs and McLane, in their work with the Bureau of Soils (2), determined that such a relation existed, but in their work the moisture retentiveness of the soil under a centrifugal force of 3000 times gravity was taken as the moisture equivalent and the effect of the organic matter in the soil was included, so the formulae derived by them were not applicable to the present work.

Briggs and Shantz (3) have derived a formula for calculating the moisture equivalent from the mechanical analysis which is as follows:

$$\text{Moisture equivalent} = 0.02 \text{ sand} + 0.22 \text{ silt} + 1.05 \text{ clay} \tag{1}$$

This formula was applied to several of the samples under consideration and found to give results which were considerably higher than the observed values, due no doubt to the high coefficient for the clay.

In order to determine whether a more satisfactory equation could be derived, an observation equation for each of the 172 samples considered was made up in the following form: x (per cent of sand) + y (per cent of silt) + z (per cent of clay) = moisture equivalent (as determined by observation). These equations were then examined by the method of least squares (4) and the values of the three unknown coefficients x, y, and z, which would most nearly satisfy the 172 observed equations, were determined, as follows: $x = 0.0627$; $y = 0.2912$; $z = 0.4257$. These values substituted in the above equation give the equation

$$0.063 \text{ sand} + 0.291 \text{ silt} + 0.426 \text{ clay} = M \tag{2}$$

where M represents moisture equivalent.

The determinations were then divided into three groups, and in a like manner an equation was derived for each group.

Group A. Soils containing less than 20 per cent silt and clay:

$$0.02 \text{ sand} + 0.40 \text{ silt} + 0.53 \text{ clay} = M \tag{3}$$

This equation was based on 15 observations.

Group B. Soils containing 20 to 50 per cent silt and clay:

$$0.02 \text{ sand} + 0.35 \text{ silt} + 0.63 \text{ clay} = M \tag{4}$$

This equation was based on 65 observations.

Group C. Soils containing more than 50 per cent silt and clay.:

$$0.11 \text{ sand} + 0.27 \text{ silt} + 0.40 \text{ clay} = M \tag{5}$$

This equation was based on 92 observations.

Groups B and C were further subdivided into their respective classes and an equation derived for each class. Group A was omitted on account of the small number of observations in each class.

TABLE 2

Calculated moisture equivalent compared with observed moisture equivalent, showing relative value of equations

SAMPLE NO.	DESCRIPTION	OBSERVED* MOISTURE EQUIVALENT	CALCULATED MOISTURE EQUIVALENT												MECHANICAL ANALYSIS		
			Equation No	M	Difference	Equation No	M	Difference	Equation No	M	Difference	Equation No	M	Difference	Sand	Silt	Clay
331760	Coarse sand.	4.30	2	9.31	5.01	3	6.57	2.27				1	8.06	3.76	89.5	5.6	4.8
242924	Sand	4.30	2	9.38	5.08	3	6.83	2.53				1	6.15	1.85	88.5	8.4	3.2
331757	Sand	5.65	2	9.38	3.73	3	7.41	1.76				1	9.59	3.94	87.2	6.6	6.1
331718	Fine sand.	7.60	2	11.10	3.50	3	9.42	1.82				1	10.85	3.25	82.4	10.8	6.5
450850	Fine sand	9.25	2	11.33	2.08	3	9.80	0.60				1	10.01	0.76	80.6	13.8	5.1
575221	Very fine sand	6.30	2	7.80	1.50	3	4.33	-1.97				1	4.44	-1.86	94.7	4.1	1.5
450872	Very fine sand.	8.20	2	11.03	2.83	3	9.51	1.31				1	9.00	0.80	81.6	14.4	4.0
242925	Sandy loam	7.70	2	12.70	5.00	4	11.89	4.19	6	11.31	3.61	1	13.01	5.31	76.7	15.9	7.6
331744	Sandy loam	15.50	2	16.46	0.96	4	17.94	2.44	6	15.17	-0.33	1	21.29	5.79	64.1	21.3	14.6
331736	Fine sandy loam	14.85	2	15.68	0.83	4	17.15	2.30	7	16.72	1.87	1	16.33	1.48	60.7	31.5	7.8
234712	Fine sandy loam	16.30	2	17.07	2.17	4	18.66	2.36	7	17.61	3.33	1	20.42	4.12	60.0	27.2	12.6
272012	Fine sandy loam	17.20	2	19.37	0.77	4	22.25	5.05	7	20.53	1.31	1	24.45	7.25	51.4	32.9	15.4
331738	Fine sandy loam	18.45	2	20.23	1.78	4	24.09	5.64	7	20.81	2.36	1	30.51	12.06	52.6	24.6	22.9
234729	Sandy clay	20.60	2	21.69	1.09	4	26.99	6.39	8	29.96	9.36	1	38.34	17.74	51.6	16.4	32.1
234713	Sandy clay	27.00	2	22.52	-4.48	4	28.74	1.74	8	31.13	4.13	1	42.96	15.96	50.4	11.7	37.5
450810	Loam	17.20	2	19.46	2.26	5	20.67	3.47	9	23.69	6.49	1	22.17	4.97	49.4	38.5	12.1
234714	Loam	18.80	2	20.33	1.53	5	21.24	2.44	9	22.50	3.70	1	22.94	4.14	45.0	42.4	12.1
272002	Loam	21.25	2	22.38	1.13	5	22.93	1.68	9	21.85	0.60	1	29.64	8.39	40.2	40.4	19.0
450833	Loam	21.65	2	22.95	1.30	5	23.28	1.63	9	25.05	3.40	1	28.43	6.78	36.9	46.6	16.6
560858	Loam	23.70	2	21.18	-2.52	5	22.05	-1.65	9	22.72	-0.98	1	27.02	3.32	44.8	38.5	16.8
234730	Silt loam	17.00	2	22.84	5.84	5	22.93	5.93	10	28.27	11.27	1	23.05	6.05	32.9	57.9	9.2
331791	Silt loam	20.10	2	23.77	3.67	5	23.64	3.34	10	27.82	7.72	1	25.01	4.71	29.9	59.4	10.8
272001	Silt loam	25.25	2	23.78	-1.47	5	23.77	-1.48	10	27.44	2.19	1	28.42	3.17	32.3	51.8	15.6
450834	Clay loam	22.90	2	23.29	0.39	5	23.62	0.72	12	23.08	0.18	1	31.41	8.51	36.9	42.2	20.4

														*			
331799	Clay loam .	23 95	2	25 52	1 57	5	25 41	1 46	12	21 89	− 2 06	1	37 87	13 92	31 1	41 9	26 7
272043	Clay loam .	24 15	2	24 72	0 57	5	24 75	0 60	12	22 06	− 2 09	1	36 12	11 97	33 9	40 7	25.2
450806	Clay loam .	30 40	2	24 92	−5 48	5	24 88	− 5 52	12	23 02	− 7 38	1	34 71	4 31	31 9	45 1	23 0
272021	Silty clay loam	25 70	2	26 04	0 34	5	25 73	0 03	13	26 13	0 43	1	35 42	9 72	27 4	50 7	22 6
331789	Silty clay loam	26 55	2	27 15	0 60	5	26 53	− 0 02	13	27 80	1 25	1	38 30	12 75	23 8	51 1	25 3
331788	Silty clay loam	29 70	2	27 60	−2 10	5	26 85	− 2 85	13	27 58	− 2 12	1	38 71	9 01	21 8	53 1	25 3
560816	Silty clay loam	29 75	2	30 75	1 00	5	28 99	− 0 76	13	27 06	− 0 69	1	42 45	12 70	7 3	64 9	26 7
234715	Clay	33 75	2	32 65	1 10	5	31 30	− 2 45	14	32 03	− 1 72	1	61 79	28 04	14 8	33 6	51 5
272018	Clay	33 95	2	29 97	−3 98	5	29 04	− 4 91	14	26 80	− 7 15	1	51 99	18 04	20 2	38 6	41 0
450858	Clay	39 35	2	29 60	−9 75	5	26 76	−12 59	14	26 44	−12 91	1	51 28	11 93	21 4	37 8	40 5
Probable error of a single determination .					±2 25			± 2 53			± 3 48			±6 76			

*Average of two determinations.

Sandy loam	9 obs.	$0.05\,\text{sand} + 0.26\,\text{silt} + 0.44\,\text{clay} = M$	(6)
Fine sandy loam	47 obs.	$0.04\,\text{sand} + 0.36\,\text{silt} + 0.43\,\text{clay} = M$	(7)
Sandy clay	9 obs.	$0.12\,\text{sand} + 0.58\,\text{silt} + 0.83\,\text{clay} = M$	(8)
Loam	24 obs.	$0.31\,\text{sand} + 0.19\,\text{silt} + 0.09\,\text{clay} = M$	(9)
Silt loam	19 obs.	$0.36\,\text{sand} + 0.26\,\text{silt} + 0.15\,\text{clay} = M$	(10)
Clay loam			
(a) Surface	5 obs.	$0.04\,\text{sand} + 0.19\,\text{silt} + 0.57\,\text{clay} = M$	(11)
(b) Subsoil	15 obs	$0.22\,\text{sand} + 0.34\,\text{silt} + 0.03\,\text{clay} = M$	(12)
Silty clay loam	12 obs.	$0.18\,\text{sand} + 0.065\,\text{silt} + 0.80\,\text{clay} = M$	(13)
Clay	17 obs.	$0.00\,\text{sand} + 0.11\,\text{silt} + 0.55\,\text{clay} = M$	(14)

In order to determine the value of these equations, 36 samples of soil were taken at random, the only considerations being that each class should be represented, and that they should be distributed as widely as possible. Two of these samples were later rejected, one because of the very high content of organic matter and the other because it puddled in the centrifuge, giving an unsatisfactory determination of the moisture equivalent.

The moisture equivalent was determined for each of the 34 samples and compared with the moisture equivalent as calculated by each of four different formulae The results are shown in table 2. The distribution of the samples was as follows:

Black Hawk, Iowa	10
Beaufort, N. C.	6
Mahoning, Ohio	6
Canadian, Okla	7
Yamhill, Ore.	2
Marlboro, S. C.	2
Brawley, Calif.	1
Total	34

The results shown in this table indicate that equation (2) gives values for the moisture equivalent which on the average more nearly approximate the observed values than any of the others. Equations (3), (4), and (5) give practically the same results. Equation (3) is to be preferred to equation (2) for the soils of Group A, as equation (2) tends to give high results in this particular group. In the other two groups there seems to be little difference, but equation (2) is probably to be preferred on account of the greater ease of manipulation.

For the rapid calculation of the moisture equivalent by means of equation (2) a logarithmic chart was prepared (fig. 1). The products of the percentages of sand, silt, and clay multiplied by their respective coefficients may be read directly from the chart as illustrated in the example, then the sum of the three terms is the calculated moisture equivalent. This chart gives results which are in as close agreement as those obtained by the use of an ordinary slide rule and for the purpose of rapid calculation is more convenient.

Equations (6) to (14) give results at slightly wider variance, which seems to be an anomaly as an equation which is derived for a particular class of soil should evidently give a more accurate result than one which embodies all classes. However, because of the smaller number of observations considered in the derivation of these equations the relation existing between the sand, silt and clay, and the moisture retentiveness is not established as closely as in the case where a larger number of observations are considered This indicates also that the relation existing is practically constant in all classes of soils—differences, if any, being due to the presence of organic matter, variations in the amount of the different grades of sand, and other factors which are not taken into consideration in the application of the equations

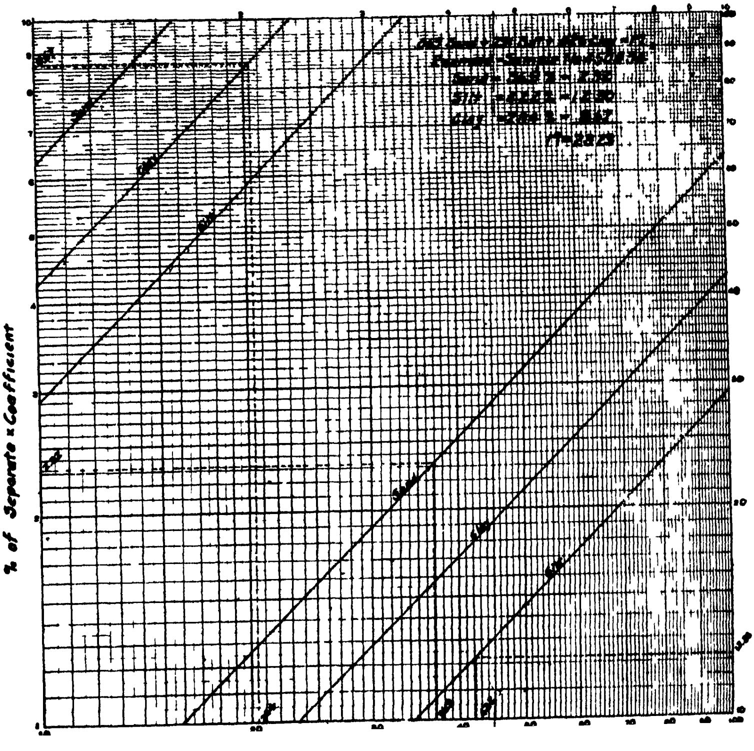

Fig 1 Logarithmic Chart for the Calculation of Moisture Equivalent

In order to determine the effect of a large amount of organic matter upon the moisture equivalent and to determine the relative value of the above equations for soils of this character, determinations were made on 30 samples of soil from Traill County, North Dakota, practically all of which were high in organic matter.

TABLE 3

Observed moisture equivalent compared with calculated moisture equivalent, showing effect of organic matter

SAMPLE NUMBER	DESCRIPTION	OBSERVED MOISTURE EQUIVALENT	CALCULATED MOISTURE EQUIVALENT					
			Equation 2	Difference	Equations 3, 4, and 5	Difference	Equation 1	Difference
351701	Fine sandy loam .	27 00	17 15	− 9 85	17 67	− 9 33	18 23	− 8 77
351702	Loam	23 85	23 24	− 0 61	21 13	− 2 72	25 52	1 67
351703	Loam .	23 45	22 67	− 0 78	22 24	− 1 21	23 67	0 22
351704	Fine sandy loam .. .	17 55	12 97	− 4 58	13 27	− 4 28	15 17	− 2 38
351705	Coarse sand.	4 90	5 01	0 11	5 01	0 11	6 02	1 12
351713	Silt loam .	33 55	26 89	− 6 66	25 23	− 8 32	28 75	− 4 80
351714	Silt loam .	29 40	27 35	− 2 05	24 37	− 5 03	28 43	− 0 97
351715	Fine sandy loam .	25 60	18 40	− 7 10	19 51	− 6 09	21 07	− 4 53
351716	Clay	37 55	28 84	− 8 71	28 41	− 9 14	53 36	15 81
351717	Fine sandy loam	15 05	11 26	− 3 79	10 79	− 4 26	11 48	− 3 57
351718	Fine sandy loam	25 75	18 90	− 6 85	19 26	− 6 49	18 55	− 7 20
351724	Fine sandy loam	26 80	20 36	− 6 44	22 70	− 4 10	26 69	− 0 11
351725	Sandy loam	26 35	17 26	− 9 09	21 20	− 5 15	27 44	1 09
351729	Loam	33 80	22 96	−10 84	21 78	−12 02	25 67	− 8 13
351730	Clay loam .	27 90	22 25	− 5 65	23 11	− 4 79	31 62	3 72
351747	Clay loam .	30 65	25 72	− 4 93	25 64	− 5 01	39 39	8 74
351748	Fine sandy loam	37 45	19 99	−17 46	21 62	−15 83	23 79	−13 66
351749	Fine sandy loam	32 85	17 52	−15 33	19 47	−13 38	22 03	−10 82
351750	Clay loam	27 30	23 16	− 4 14	23 56	− 3 74	31 69	4 39
351751	Fine sandy loam	22 35	13 63	− 8 72	13 32	− 9 03	14 01	− 8 34
351752	Fine sandy loam	18 90	13 47	− 5 43	13 27	− 5 63	15 76	− 3 14
351753	Loam	27 50	22 85	− 4 65	23 31	− 4 19	30 68	3 18
351754	Fine sand	12 70	11 32	− 1 38	9 69	− 3 01	11 46	− 1 24
351755	Fine sand.	8 35	9 93	1 58	7 49	− 0 86	9 39	1 04
351756	Clay loam .	34 45	24 03	−10 42	24 32	−10 13	34 55	0 10
351757	Clay loam .	36 00	24 39	−11 61	24 54	−11 46	36 40	0 40
351758	Fine sandy loam	28 65	16 45	−12 20	17 83	−10 82	20 51	− 8 14
351759	Sandy clay	21 50	22 71	1 21	28 99	7 49	43 72	22 22
351760	Loam	38 20	21 26	−16 94	22 14	−16 06	28 01	−10 19
351761	Clay loam .	29 50	26 37	− 3 13	26 41	− 3 09	40 67	11 17
Probable error of a single determination .				± 5 65		± 5 44		± 5.32

Briggs and McLane (2) determined that organic matter has practically the same effect as an equal amount of clay in increasing the moisture equivalent. This fact is borne out in the results of the determinations as shown in table 3. In nearly every case the observed was higher than the calculated value. The results also indicate that with soils of this character the indirect determination is not as reliable as with ordinary soils, the probable error of a single determination being approximately twice as great. It is interesting to note in this connection that equation (1), which gives results at widest variance, as shown in table 2, gave better results with soils of this character and had the least probable error. The similarity of the results of equation (2) and equations (3), (4), and (5) are borne out also, the latter in this case having a slight advantage.

SUMMARY

The moisture equivalent may not be used as a basis for the classification of soils, but may be of valuable assistance in the interpretation of the mechanical analysis.

There is a direct relationship between the miosture equivalent and the percentages of sand, silt, and clay in the soil as determined by mechanical analysis. This relation may be expressed as

$$0.063 \text{ sand} + 0.291 \text{ silt} + 0.426 \text{ clay} = \text{moisture equivalent}$$

The presence of considerable amounts of organic matter in the soil tends to increase the moisture equivalent and to disturb the relation between the moisture equivalent and the mechanical analysis.

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THE INFLUENCE OF SAND UPON THE CONCENTRATION AND REACTION OF A NUTRIENT SOLUTION FOR PLANTS

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Finely divided solid substances of a certain character presenting large surfaces appear to have the power of removing salts from solution by concentrating the solute at the surface of the solid material. It has thus been shown that soils not only have the power to adsorb salts as such from solution but that this adsorption may become selective in character in that one kind of ion may be removed from solution at a more rapid rate than another kind.

Parker (6) has measured the adsorbing power of soils and has shown that the concentration of salts may be altered in contact with the soil particles. He states that a mineral fertilizer applied to the soil and dissolved in the soil solution is adsorbed either as a whole or selectively from the solution by the great surface presented by the finely divided soil particles, and is held there until the plant or subsequent leaching removes it. McCall (5) grew wheat in sand cultures and found the responses of the plants to the nutrient solutions in the sand to be different from those of similar wheat plants grown in water cultures in which the same solutions were employed. This author suggests that the salt proportions and total concentrations of the nutrient solutions may have been altered in contact with the sand particles and concludes that differential adsorption played an important rôle in bringing about the observed physiological differences. Jennings (3) found that the introduction of quartz, ferric hydroxide, and aluminum hydroxide into nutrient solutions resulted in decreased growth of wheat seedlings, and from this concludes that these substances by their adsorptive properties reduced the effective concentrations of the nutrient solutions. On the other hand, Toole and Tottingham (9), growing barley and pea plants for 21 days in Knop's nutrient solution to which had been added separately ferric hydrate, silicic acid, and carbon black, found no clear evidence of the adsorption of nutrient ions from the solutions, although their results are based upon careful chemical analyses of the treated solutions as well as upon the effect of the added solids on the growth of the plants either directly of indirectly. In a study of the effect of ammonium sulfate in nutrient solutions upon the growth of plants with sand as the solid substratum to which the solutions were added, Wolkoff (10) pointed out that the concentration values of the solutions obtained by suction from the sand in the culture pots at different times during the growth period did not vary considerably from those of the original solutions. This author concluded that adsorption did not modify the concentrations of the solutions to such an extent as to produce even a slight influence upon plant growth.

In the study of the salt requirements of plants in sand cultures it is, of course, desirable to maintain approximate control of the salt proportions and the effective concentration of the nutrient solution applied to the sand. This is one of the chief reasons for resorting to sand cultures in investigations of this kind, since agricultural soils, when employed as substrata, always present chemical and biological complications which render adequate control of these important factors exceedingly difficult, if not impossible. It is generally considered that sand cultures present somewhat similar physical environmental conditions to those found in soil cultures, but plants grown in sand cultures are relatively free from the biological complications such as are introduced when agricultural soils are used.

It is possible that nutrient solutions supplied to sand in pot cultures may suffer not only a reduction in total concentration but also a change in the relative proportions of the constituent salts and ions as a result of contact with the solid sand particles through the adsorptive action of the solid medium. However, if ions or salts as a whole are thus removed from solution in sufficient quantities to alter the growth rates of the plants materially, it should be possible to detect the change in total concentration of the solution. A change in the reaction of the solution might also be expected when salts are removed from solution in quantities sufficient to affect the growth rates of the plants, since it is not probable that the salts or ions would be adsorbed in the same proportions in which they occurred in the solution.

The purpose of the experiments described in the following pages was to study the influence of washed sand of several degrees of fineness, and also of very fine unwashed sand, upon the effective concentration and reaction of a nutrient solution diffused as films on the solid sand particles, when the cultures were subjected to the treatment experienced in the usual course of sand culture work with frequent renewal of solutions, but in the absence of plants—since contact with the roots of actively growing plants may change the reaction of a nutrient solution very materially, especially if the contact is long continued.

METHODS OF INVESTIGATION

White seashore sand was used throughout these experiments. For the first washing the sand was placed in a graniteware tub, a little more than half filling the latter. A stream of tap water from a hose was directed into the sand, which was kept constantly agitated, until the surplus water overflowing the sides of the tub was clear and entirely free from sediment. The tap water was then drained off and the tub nearly filled with distilled water in which the sand was thoroughly washed. The wash water was drained off and the process once repeated. The washed sand was spread on large sheets of paper and allowed to become air-dry.

The air-dry sand was passed through sieves and four grades with respect to sizes of the particles were obtained. The sizes of the particles of the four different grades obtained by this mechanical separation were approximately as follows: very coarse sand, 1.5 to 1 mm.; coarse sand, 1 to 0.5 mm.; medium sand, 0.5 to 0.32 mm.; and fine sand, 0.32 to 0.25 mm. The water-retaining capacity of these four grades of sand determined according to the method described by Hilgard (2), and the values referred to the dry weight of the sand were, respectively, 26.8 per cent, 30.2 per cent, 32.4 per cent, and 37.6 per cent. These values represent the averages of four determinations for each grade.

Twelve hundred grams of air-dry sand of each of the four grades were weighed into 1-liter stoneware pots impervious to moisture, three pots to each grade of sand. The sand in each pot was then flooded with a nutrient solution for plants (solution R_6C_2 of Shive's (7) 3-salt series) of the following composition: KH_2PO_4, 0.0180m.; $Ca(NO_3)_2$, 0.0052m.; and $MgSO_4$, 0.0150m. The excess solution was drawn off through a glass tube extending through the sand to the bottom of the pot, the lower opening of the tube being screened by a plug of glass wool which served as a filter. A sufficient amount of solution was thus drawn off to leave the moisture content of the sand at 60 per cent of its water-retaining capacity in every case. Evaporation from the surface of the sand was prevented by placing the pots under small bell jars which were then sealed.

These cultures received subsequent treatment similar to that accorded to sand cultures with growing plants in the usual course of sand culture work, with renewal of solutions by the McCall (4) method at intervals throughout the experimental period. During the first interval the cultures were allowed to stand for 24 hours. At the end of this period as much as possible of the old solution was extracted, after which each culture was flooded with 250 cc. of new solution, the excess of which was again drawn off leaving the sand with its original moisture content. This process was repeated at intervals of 3, 5, and 10 days, the total experimental time covering a period of 19 days. The old solutions extracted from the cultures at the end of each interval were carefully tested for freezing-point lowerings and for hydrogen-ion concentrations. These results were compared with those of similar tests of the original solution made at the beginning of each interval.

The freezing-point depressions of the solutions were determined by the use of the Beckmann apparatus and from these the total osmotic concentration values in atmospheres were calculated after the proper corrections were made for the undercooling of the solutions. The hydrogen-ion concentration of the solutions was determined by the colorimetric method following the work of Clark and Lubs (1) as a guide in the preparation of the standard buffer solutions and in the general methods of procedure. Methyl red was found to be a satisfactory indicator for the solutions here tested.

RESULTS WITH WASHED SAND

Table 1 presents the experimental results obtained with the four different grades of washed sand. Corresponding data for very fine unwashed sand containing relatively large amounts of colloidal or semi-colloidal material will be given in another table.

In table 1 are given the hydrogen-ion concentrations (expressed as pH values) of the nutrient solutions both before and after having been diffused as films over the solid sand particles. The freezing-point depressions, corrected for undercooling, and the corresponding derived osmotic concentration values for 25°C. (measured in atmospheres) are given. The last column of the table shows the plus or minus per cent deviations from the osmotic concentration values of the original solution. The table is divided into four horizontal sections, each of which presents the complete data obtained with a single grade of sand.

It will be observed that the solutions extracted from the different grades of sand at the end of each time interval all show very close agreement with each other and with the original solution with respect to the pH values. The pH value of the original solution was 4.7 in all cases while the average pH values of the extracted solutions ranged between 4.7 and 4.8, representing hydrogen-ion concentrations not higher than 0.200×10^{-4} (pH 4.7) nor lower than 0.159×10^{-4} (pH 4.8). Three of the average pH values of the extracted solutions are equal to that of the original solution. These three are shown for the solutions extracted from the very coarse sand at the end of the 10-day interval, and for those from the medium sand at the end of the 24-hour and 10-day intervals. All the other average pH values are slightly above that of the original solution. None is lower than that of the original solution.

The fact that nearly all the average pH values are slightly above and none below that of the original solution might be regarded as indicating a slight reaction change of the nutrient solution resulting from contact with the solid sand particles. However, the very small deviations of the average pH values from that of the original solution, and the correspondingly slight reaction changes of the nutrient solution represented by them must be regarded as being without significance when considered in connection with any influence which they might have upon the growth rates of plants.

The average total concentration values (in atmospheres) of the solutions extracted from the different grades of sand, like the pH values of these solutions show very close agreement with the corresponding values of the original solution. The highest total concentration value (average of three tests) was 1.80 atmospheres, representing a deviation of 2.86 per cent above that of the original solution. This is shown for the solutions extracted from the very coarse sand at the end of the 24-hour interval. The lowest average concentration value was 1.74 atmospheres, which is 1.14 per cent below that shown for the original solution. This was the average concentration for the solutions

TABLE 1

Data for a nutrient solution before applying to and after extracting from washed sand of several degrees of fineness

DESCRIPTION OF SAND	TIME SOLUTION REMAINED IN CONTACT WITH SAND WITHOUT RENEWAL	CULTURE NUMBER	HYDROGEN-ION CONCENTRATION	FREEZING-POINT DEPRESSION	OSMOTIC CONCENTRATION VALUE AT 25°C.	PLUS OR MINUS DEVIATION FROM CONCENTRATION VALUE OF ORIGINAL SOLUTION
			pH*	°C.	*atm.*	*per cent*
Very coarse sand, 1.5 to 1.0 mm.	24 hours	1	4.7	0.133	1.75	0.00
		2	4.8	0.138	1.81	+3.43
		3	4.8	0.139	1.83	+4.57
		Average	**4.77**	**0.137**	**1.80**	**+2.86**
	3 days	1	4.8	0.133	1.75	0.00
		2	4.8	0.135	1.77	+1.14
		3	4.8	0.133	1.75	0.00
		Average	**4.8**	**0.134**	**1.76**	**+0.57**
	5 days	1	4.8	0.134	1.76	+0.57
		2	4.7	0.136	1.78	+1.71
		3	4.8	0.132	1.74	−0.57
		Average	**4.77**	**0.134**	**1.76**	**+0.57**
	10 days	1	4.7	0.133	1.75	0.00
		2	4.7	0.132	1.74	−0.57
		3	4.7	0.134	1.76	+0.57
		Average	**4.7**	**0.133**	**1.75**	**0.00**
		Original solution	4.7	0.133	1.75	
Coarse sand, 1.0 to 0.5 mm	24 hours	4	4.7	0.131	1.72	−1.71
		5	4.8	0.135	1.77	+1.14
		6	4.7	0.140	1.84	+5.14
		Average	**4.73**	**0.135**	**1.78**	**+1.71**
	3 days	4	4.8	0.140	1.84	+5.14
		5	4.7	0.130	1.71	−2.29
		6	4.8	0.132	1.74	−0.57
		Average	**4.77**	**0.134**	**1.76**	**+0.57**
	5 days	4	4.7	0.133	1.75	0.00
		5	4.7	0.135	1.77	+1.14
		6	4.8	0.133	1.75	0.00
		Average	**4.73**	**0.134**	**1.76**	**+0.57**
	10 days	4	4.8	0.136	1.78	+1.71
		5	4.7	0.137	1.80	+2.86
		6	4.8	0.134	1.76	+0.57
		Average	**4.77**	**0.136**	**1.78**	**+1.71**
		Original solution	4.7	0.133	1.75	

TABLE 1—*Concluded*

DESCRIPTION OF SAND	TIME SOLUTION REMAINED IN CONTACT WITH SAND WITHOUT RENEWAL	CULTURE NUMBER	HYDROGEN-ION CONCENTRATION	FREEZING-POINT DEPRESSION	OSMOTIC CONCENTRATION VALUE AT 25°C.	PLUS OR MINUS DEVIATION FROM CONCENTRATION VALUE OF ORIGINAL SOLUTION
			pH*	°C	*atm.*	*per cent*
Medium sand, 0.5 to 0.32 mm.	24 hours	7	4.7	0.135	1.77	+0.57
		8	4.7	0.144	1.89	+7.39
		9	4.7	0.133	1.75	−0.57
		Average	**4.7**	**0.137**	**1.80**	**+2.27**
	3 days	7	4.8	0.133	1.75	−0.57
		8	4.8	0.131	1.72	−2.27
		9	4.7	0.133	1.75	−0.57
		Average	**4.77**	**0.132**	**1.74**	**−1.14**
	5 days	7	4.8	0.135	1.77	+0.57
		8	4.7	0.135	1.77	+0.57
		9	4.7	0.133	1.75	−0.57
		Average	**4.73**	**0.134**	**1.76**	**0.00**
	10 days	7	4.7	0.134	1.76	0.00
		8	4.7	0.130	1.71	−2.84
		9	4.7	0.133	1.75	−0.57
		Average	**4.7**	**0.112**	**1.74**	**−1.14**
		Original solution	4.7	0.134	1.76	
Fine sand, 0.32 to 0.25 mm.	24 hours	10	4.7	0.135	1.77	+1.14
		11	4.8	0.135	1.77	+1.14
		12	4.8	0.131	1.72	−1.71
		Average	**4.77**	**0.134**	**1.76**	**+0.57**
	3 days	10	4.7	0.132	1.74	−0.57
		11	4.8	0.133	1.75	0.00
		Average	**4.75**	**0.133**	**1.75**	**0.00**
	5 days	10	4.8	0.134	1.76	+0.57
		11	4.8	0.134	1.76	+0.57
		12	4.8	0.133	1.75	0.00
		Average	**4.8**	**0.134**	**1.76**	**+0.57**
	10 days	10	4.7	0.134	1.76	+0.57
		12	4.8	0.136	1.76	+1.71
		Average	**4.75**	**0.135**	**1.77**	**+1.14**
		Original solution	4.7	0.133	1.75	

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* The term pH is given to the number representing the logarithm of the hydrogen-ion concentration taken as a positive value.

extracted from the medium sand at the end of the 3-day and 10-day intervals. All but five of the average concentration values represent plus deviations from the concentration of the original solution and only two represent minus deviations. There is no regularity in the manner in which these deviations occur and all are very small so that no significance can be attached to them, especially when considered in relation to the relatively low sensitiveness of ordinary plants to slight variations in the total salt concentrations of the medium in which they are grown. Thus, with the nutrient solution here employed, with sand of different degrees of fineness, washed free from colloidal or semi-colloidal material, there is no clear evidence of the adsorption of salts or ions in sufficient amounts to alter materially the hydrogen-ion concentration of the nutrient solution or to reduce its effective total concentration measurably. This is in accord with the results of previous work (8) with washed sand, in which it was found that the physiological properties of nutrient solutions as these affected the growth rates of buckwheat plants were not materially altered as the result of direct contact with the solid sand particles.

RESULTS WITH UNWASHED SAND

The unwashed sand here employed was taken from the same supply of white seashore sand as was the washed sand used in the preceding experiments. The coarser particles of the sand were mechanically separated from the finer by means of a sieve which permitted only particles with diameters less than 0.25 mm. to pass, the coarser sand being discarded. This very fine sand contained relatively large quantities of colloidal or semi-colloidal material, as is indicated by the fact that when 100 gm. of the sand were shaken with 250 cc. of distilled water for one minute and then allowed to settle for 30 minutes, 100 cc. of the supernatant liquid contained 0.260 gm. of suspended solid material, and traces of the suspended material were still present in the supernatant liquid at the end of a week. The water-retaining capacity of this fine sand was 39.87 per cent of the dry weight of the sand.

The methods used with this fine unwashed sand were identical with those adopted in the preceding experiments with washed sand. The nutrient solution added to the sand was the same in composition and total concentration as that previously employed and this was renewed at intervals of 24 hours, 2 days, 4 days, and 3 days, covering a total experimental period of 10 days.

As before, 1200 gm. of sand were used with each culture. The series consisted of four cultures which received similar treatment, and fifth, control culture, to which distilled water was applied instead of the nutrient solution, in all other respects receiving the same treatment as the other cultures. The control culture was included in the series in order to determine to what extent the freezing point might be depressed and the total concentration of the nutrient solution correspondingly increased by soluble substances in the sand. In this it is assumed, of course, that the efficiency of the nutrient solution as a

TABLE 2

Data for nutrient solution before applying to and after extracting from very fine unwashed sand

TIME SOLUTION REMAINED IN CONTACT WITH SAND WITHOUT RENEWAL	CULTURE NUMBER	HYDROGEN-ION CONCENTRATION	FREEZING-POINT DEPRESSION	OSMOTIC CONCENTRATION VALUE AT 25°C.	PLUS OR MINUS DEVIATION FROM CONCENTRATION VALUE OF ORIGINAL SOLUTION
		pH	°C.	*atm.*	*per cent*
24 hours	1	4 9	0.122	1 61	−8.52
	2	4 8	0 121	1.59	−9 66
	3	4 7	0 123	1 62	−7 95
	4	4 7	0 124	1 63	−7 38
	Average	**4 77**	**0 123**	**1.61**	**−8 50**
	Original solution	4 7	0 134	1.76	
2 days	1*				
	2	4 7	0 131	1 73	−1 14
	3	4 8	0 129	1 70	−2 86
	4	4 8	0 130	1 71	−2 28
	Average	**4 76**	**0 130**	**1 71**	**−2 29**
	Original solution	4.7	0 133	1 75	
4 days	1				
	2	4 7	0 133	1 75	0.00
	3	4 7	0 132	1 74	−0 57
	4	4 7	0 135	1 78	+1 71
	Average	**4 7**	**0 133**	**1 75**	**+0 57**
	Original solution	4 7	0 133	1 75	
3 days	1				
	2	4 8	0 133	1 75	0 00
	3	4 7	0 132	1 74	−0 57
	4	4 7	0 134	1.76	+0 57
	Average	**4 73**	**0 133**	**1 75**	**0.00**
	Original solution	4 7	0 133	1 75	

* Through accident it became necessary to discontinue culture 1, at the end of the first interval.

solvent of the mineral constituents of the sand was equal to that of distilled water.

The numerical data of the tests with unwashed sand here used are presented in table 2. The data of this table correspond to those of table 1. The freezing-point lowerings, corrected for undercooling, as given in table 2, are the values obtained after deducting the freezing-point depression resulting from soluble substances in the sand, determined by tests made of the extract from the sand of the control culture at the end of each interval. Thus, at the end of the first interval the extract from the control culture gave a freezing-point lowering of 0.018°C. (average of four tests) corrected for undercooling. This was then deducted from the freezing-point depressions of the solutions extracted from the sand of the other cultures, giving the values as they appear in the table for the interval in question. This was done for each succeeding interval. At the end of the second interval the freezing-point lowering of the extract from the control culture was 0.004°C. (average of four tests), but at the end of the third interval no depression of the freezing point, below that of the distilled water used, could be detected. Thus, while the sand at first contained small quantities of soluble matter, this was removed with the successive renewals of the distilled water in the control culture, so that after the second renewal no more soluble substance remained, in so far as this could be detected by means of the freezing-point method.

Inspection of table 2 shows the pH values of the solutions extracted from the sand to be in very close agreement with those of the original solution. Three of the four average pH values are slightly higher and one is equal to that of the original solution. As in the preceding experiments with washed sand there is here no evidence of marked change in the reaction of the nutrient solution which might be attributed to adsorption phenomena resulting from direct contact of the solution with the solid sand particles.

The solutions extracted from the sand at the close of the first and second intervals show consistently lower total concentration values than do those of the original solution. The average of these values for the first interval is 1.61 atmospheres, representing a deviation of 8.50 per cent below that of the original solution. The corresponding average for the solutions at the end of the second interval is 1.71 atmospheres, representing a minus deviation of 2.29 per cent, but at the end of the third interval the average total concentration of the extracted solution is 1.76 atmospheres, this being a deviation of 0.57 per cent above that of the original solution.

The fact that the total concentration values of the solutions extracted from the sand at the end of the first and second intervals are consistently lower than that of the original solution, is fairly clear evidence that the adsorptive capacity of the sand is here effective in reducing the total concentration of the solution, although this reduction was not large even during the first interval. With the second renewal of solution at the beginning of the 4-day interval, it appears that equilibrium was established with respect to the adsorptive capacity of the sand, that is, the ability of the sand to adsorb salts from this solution had reached its limit, therefore no reduction in the total concentration occurred when the solution was again renewed.

As already brought out, the results obtained with washed sand gave no evidence of any reduction in the total concentration of the nutrient solution applied to the sand which might be attributed to adsorption. The observed reduction in the total concentration of the solution added to the unwashed sand here used is, therefore, to be ascribed to the adsorptive action of the finely divided, colloidal or semi-colloidal material which was removed from the sand in the process of washing.

The greatest reduction in the total concentration observed is 0.17 atmospheres, representing a minus deviation of 9.66 per cent of the concentration of the nutrient solution added to the sand. Considering the relatively low sensitiveness of agricultural plants to slight variations in the total salt concentration of the medium in which they are grown, even such a reduction in the total concentration of a nutrient solution when added to sand in sufficient quantities to give optimum moisture content, is scarcely sufficient to produce any marked changes in the growth rates of plants so long as the concentration is well within the range required for good growth and the solution is frequently renewed by some method such as that devised by McCall (4), or by some other adequate method. If, on the other hand, the total concentration of the nutrient solution added to the sand should be considerably below that required for approximately optimum growth it is readily to be seen that even a slight reduction in the effective concentration, or a change in the salt or ion proportions through differential adsorption might have a very marked effect upon the growth of plants already suffering from an inadequate salt supply.

Perhaps the most noteworthy feature brought out in these tests is the fact that by removal from the sand of the very finely divided colloidal or semi-colloidal material by thorough washing in flowing water which carried away this material in suspension, the power of the sand to adsorb salts from the nutrient solution used was eliminated, in so far as any change in total concentration or reaction could be detected by the methods here employed. It thus appears that in culture work with washed sand, alteration of the physiological properties of nutrient solutions (such as was here used) by the adsorptive properties of the sand, sufficient to influence the growth rates of plants, is not to be expected when the solutions are diffused as films on the solid particles of the substrata. It further appears that frequent renewal of solutions will soon overcome any initial adsorptive effects of very fine sand containing considerable quantities of colloidal or semi-colloidal material, by saturating the adsorptive capacity of the sand

SUMMARY

A study was made of the influence of washed sand of four different degrees of fineness, and also of very fine unwashed sand, upon the reaction and concentration of a nutrient solution. The solution was applied to the sand in culture pots to give a moisture content of 60 per cent of the water-retaining capacity of the sand in every case. The solution was renewed at stated intervals after as much as possible of the old solution had been extracted from the sand by suction. Tests were made of these solution samples for the hydrogen-ion concentration and for the total salt concentration and the results of these tests were compared with those of similar tests of the original solution.

1. With the different grades of washed sand there was no evidence of the adsorption of salts or ions in sufficient amounts to alter materially either the reaction or the total salt concentration of the solution in contact with the solid particles of the substrata.

2. The adsorptive capacity of the unwashed sand was sufficient to reduce the total salt concentration of the solution from 1.76 atmospheres to 1.61 atmospheres (average reduction of 8.50 per cent) during the first 24-hour interval.

3. The reaction of the nutrient solution was not markedly altered by contact with the unwashed sand.

4. By renewal of the nutrient solution the initial adsorptive effect of the unwashed sand was soon eliminated, apparently by saturating its adsorptive capacity.

5. The adsorptive properties of the unwashed sand appeared to be due to the very finely divided colloidal or semi-colloidal material which was removed from the sand in the process of washing.

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EXPERIMENTS ON THE VALUE OF COMMON ROCK SALT AND SULFUR FOR KILLING LIVE STUMPS[1]

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INTRODUCTION

The clearing of land in New Jersey, where great tracts of valuable land are covered with pine and oak woods, is commonly accomplished both by the method of "suckering" and with the help of goats. Suckering is tedious and expensive. A woodlot which is to be cleared is usually cut down close to the ground and the brush piled in large heaps and burned at the place. The next spring the "suckers" start and grow vigorously, because the live stumps have an extensive root system which enables them to send out new shoots over and over again. During the spring and summer these stumps are "suckered" two or three times. This suckering consists of breaking down as many as possible of the new shoots, and woodcutters believe that this is especially effective during the month of August. However, the other work on the farm during this month does not always allow the men to do this work as thoroughly as it should be done to make it effective. Besides, great numbers of branches cut down even in August make new sprouts again the following spring. It therefore often takes several years before the land is brought into such a shape that these stumps do not hamper the crops growing among them. The dead stumps are removed later when the time is opportune. Clearing with the help of goats proceeds in the same way, except that the thick woodlots are often first "opened up" by the goats before the tall trees and heavy underbrush are cut down. Later the suckers are kept down by the grazing goats.

To find a means whereby the clearing would be less costly and whereby the land could be improved, possibly after one or two years, was the object of the work recorded below. In order to study the effects of salt and sulfur in killing live stumps, a number of woodlots in different parts of the state of New Jersey were selected. The most extensive of these were at New Brunswick, Bridgeton, and New Lisbon; the first one being on the College Farm, the second on the farm of Mr. L. H. Sharp at Bridgeton, and the third at the Four

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[1] This work was suggested by Dr. Jacob G. Lipman, director of the New Jersey Agricultural Experiment Station, and started in cooperation with the Forest Service of the State Department of Conservation and Development.

Mile Colony of the Burlington County Farm for feeble-minded children.[3] The experiments therefore, were carried out under somewhat different climatic and soil conditions. The soil at New Brunswick consists of a mixture of Sassafras loam and Pennsylvania loam, and is rather swampy. The soil at Bridgeton is a sandy loam, and that at New Lisbon is a dry sandy soil. The experiments were started early in the spring of 1919. Other experiments, to determine whether there is a pronounced difference due to seasonal influences, were started during the year.

EXPERIMENTS WITH SALT ON A SWAMPY SOIL NEAR THE AGRICULTURAL COLLEGE AT NEW BRUNSWICK

Twelve plots, with from fifteen to seventeen good-sized stumps, were marked off. The species of tree stumps were oak (black and white), birch, maple, dogwood, chestnut, and cherry. The height of the sprouts in the spring varied from 10 to 18 feet. The amounts of salt applied on the various plots were as follows:

PLOT NUMBER	SALT PER ACRE	PLOT NUMBER	SALT PER ACRE
	lbs.		*lbs*
101	420	107	2500
102	840	108	3000
103	1250	109	3350
104	1650	110	3750
105	2100	111	4200
106	2100	112	4200

The applications were made on or around the stumps, except on the two plots (106 and 112) receiving applications of 2100 and 4200 pounds per acre, respectively, which were scattered broadcast. According to analyses made, the common rock salt which was used contained 97 to 98 per cent sodium chloride, a little magnesium chloride, and a few impurities. The salt was applied on April 3, 4 and 5, after several days of dry weather. Heavy rains during the next few days dissolved a great deal of the salt, but the remainder formed a crust which required a long time to dissolve. Even in midsummer, traces of the salt could be found under the trees which had made a more or less vigorous growth. The luxuriant foliage of the oak trees, especially, prevented dissolving.

The tree stumps were examined frequently during the summer months. Up to the last of July not much effect could be detected. An examination made in the middle of August gave the results as recorded in table 1. A later examination, made on September 5, showed that on plot 105 a small birch had died. Some of the higher branches of small maples were dying but the roots had sent out vigorous new shoots.

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[3] The writer is indebted to Mr W. M Baker, assistant forester of the State Forest Department, and to Mr. W W. Oley, county agent for Cumberland County, for their cheerful help in the laying out of the plots and the applying of the salt and sulfur; also to Mr. L. H. Sharp for placing the land at our disposition.

The effect of the salt upon these stumps of high trees, with their vigorously growing shoots, was very limited in this swamp. The larger stumps were only slightly, if at all, injured, and but few of the smaller stumps were killed. The resistance of the stumps to treatment with salt was apparently in the order of oak, cherry, chestnut, dogwood, birch, maple, the last named being affected most intensely and most easily. This would be expected if the hardiness of the wood is taken into consideration.

TABLE 1

Effect of salt treatment as recorded in the middle of August

PLOT NUMBER	HEIGHT OF SPROUTS	SALT APPLIED PER ACRE	CONDITIONS NOTED
	feet	*lbs*	
101	10 to 18	400	Oaks have deep blue-green leaves and appear abnormally vigorous. Birch thrifty. Chestnut healthy
102	9 to 16	800	Oak, maple, and cherry healthy. Oak leaves not so blue-green as on plot 101, but seem to be larger
103	9 to 15	1250	Oak, birch, maple, and dogwood healthy. Nothing unusual to be detected
104	9 to 15	1675	Oak and maple healthy. Birch slightly injured; birch leaves have brown edges
105	9 to 16	2100	Oaks healthy. Maples injured; leaves are small and shriveled. Some birch slightly injured
106	8 to 15	2100*	Small maple killed. Dogwood injured. Some birch slightly injured, others healthy. Some oaks very slightly injured, others healthy
107	9 to 15	2500	Small maple killed. One birch killed, others injured. Dogwood badly injured. Oaks healthy
108	9 to 16	3000	A few maples and birch killed, others injured. Cherry slightly injured. Oaks healthy
109	8 to 16	3400	Birch and dogwood injured, leaves turning yellow and falling. Small maple killed. Large maple injured. Oaks healthy
110	9 to 15	3800	Birch and dogwood injured. Small maple killed. Oaks healthy
111	9 to 16	4200	Maple killed. Some birch injured, others killed Pear and cherry killed. Oaks healthy. None of the large stumps killed, only slightly injured or healthy
112	9 to 16	4200*	Small maple killed, others injured. Dogwood injured. Some birch injured. No effect on oaks to be detected

* Scattered broadcast.

EXPERIMENTS WITH SALT AND SULFUR

The experiments at Bridgeton were conducted on land to be cleared for truck farming. The soil is a sandy loam. The treated plots were approximately $\frac{1}{17}$ acre, with check plots four times as large at either end of the group of treated plots. The land was cleared in the same way as is done by the practical farmer: the trees and brush were cut down and the branches burned on the ground. A counting made of the number of stumps on these plots placed the average at 45 good-sized stumps. The very small stumps were not included. Nearly all the stumps were black and white oak. About a week after the brush was burned, the salt and sulfur were applied in the following amounts:

	SODIUM CHLORIDE (POUNDS PER ACRE)			SULFUR (POUNDS PER ACRE)			
	Applications made May 15, 1919, on or around the stumps						
Check plots	*No. 1* 1000	*No. 2* 1600	*No. 3* 2100	*No. 13* 90	*No. 14* 180	*No. 15* 350	Check plots
	No. 4 2600	*No. 5* 3200	*No. 6* 3750	*No. 16* 500	*No. 17* 700	*No. 18* 850	
	No 7 4200	*No. 8* 4750	*No. 9* 5300	*No 19* 1000	*No. 20* 1300	*No. 21* 1725	
	Applications made May 15, 1919, scattered broadcast						
Check plots	*No. 10* 3200	*No. 11* 4200	*No. 12* 5300	*No. 22* 750	*No. 23* 1000	*No 24* 1500	Check plots

An examination made on July 2 showed that the growth of the suckers on plots 1, 2 and 3 was markedly retarded, while the suckers on the stumps of plots 4, 5, 6 and 7 were not only retarded, but injured and in some cases killed. On the two plots where the heaviest applications had been made, plots 8 and 9, nearly all suckers were killed, and those left on the stumps had a sickly appearance. Very small stumps, apparently not touched by the salt when the applications were made, showed a healthy growth on these plots.

The plots on which application had been made broadcast, plots 10, 11 and 12, were also affected. The huckleberry brush was killed, and some of the suckers which remained on the stumps had a sickly appearance; but the effect was by no means so marked as on the plots which received the same amount of salt, applied on and around the stumps. A very marked line was to be seen between the check plots and the treated plots, even at places where the smallest applications were made. The height of the suckers was much less on the treated plots, and the number markedly decreased.

An examination made on September 26 showed that oaks which were still growing on plots 7, 8 and 9 looked sickly. Their branches were much shorter and the leaves were small. The same was observed on the plots where salt was scattered broadcast (plots 10, 11 and 12), while the growing branches on the stumps on plots 1 to 6, where the smallest amounts were given, had a sickly appearance. In general, the leaves of the oaks on all treated plots were brownish and partly withered, while the leaves of the oaks on the check plots appeared to be healthy, with no signs of autumn to be detected as yet. On the treated plots much mildew was found, but on the check plots very little. The same was observed on a cutover land of about 15 acres where the stumps were allowed to grow throughout the entire year; here the leaves were only slightly attacked by the mildew. The sickly leaves which had brown edges and were shriveled or curled, dropped from the branches when slightly touched. The branches themselves were easily removed from the stumps when pushed down slightly, or when touched by walking past. This was not the case with branches on stumps on the check plots. These easily removable branches had a bluish black pith, indicating toxicity. This bluish black color in the center of the stems gradually decreased toward the top of the shoot, the darkest color being nearest the stump. Laboratory and other field experiments seem to indicate that when the sodium chloride is dissociated by the soil moisture and rain-water, the chlorine ion is taken up by the roots, causing toxicity. This chlorine is distributed throughout the plants, carried to the extreme end of the tracheids in the leaves or to the ends of the secondary veins, causing the brown discoloration of the leaves.

Examinations at the same time of the plots on which sulfur was applied, showed that even at the end of the season a part of the sulfur had not dissolved. Where heavy applications were made (plots 19, 20, 21, 23 and 24) the growth appeared to be slightly retarded, while on the plots where small applications were made a slight stimulating effect was noticeable. A comparison made between the plots which had received 1000 and 1600 pounds of salt per acre and the plots which had been given at least the same amount of sulfur showed that the retarding effect of the salt was markedly greater. In fact the only difference which was to be seen between the plots with the heaviest sulfur applications and the check plots, was that on the former the average length of the branches of the oak stumps appeared slightly shorter than on the latter. On a number of stumps in the plots which had received the heaviest sulfur applications it was found that some of the leaves on the lower parts of the stems had wilted and some had dropped off. Mildew had attacked the leaves on these plots much more severely than on the check plots, but much less than on the salt plots.

A series of experiments were conducted in exactly the same way near New Lisbon. Here, however, the brush from 13 to 18 feet high was not cut down, but the applications were made without disturbing the growth of the stumps. Examinations made during the summer and fall showed that the influence of the salt was very slight. No injurious effect upon the growth could be observed, but where the smallest applications were made a more or less marked stimulating effect appeared. The leaves of the high branches of the stumps on these plots were more glossy, darker in color, and larger. Where the heaviest applications were made, only slight discoloration of the leaves occurred, not enough to prevent, or even retard the growth of the stems. In the case where sulfur was applied the majority of the stumps seemed to be stimulated rather than retarded in every instance. Not all of the sulfur was dissolved when the last examination was made.

A number of experiments of a similar nature were conducted throughout the state of New Jersey to determine the most opportune time of application. Salt and sulfur applications which were made in the summer did not dissolve rapidly enough to be toxic, while applications made in early fall, when the stumps had ceased growing, had little or no effect.

CONCLUSIONS

Application of sulfur on high or low oak brush, as a means of clearing land for agricultural purposes, is of no value. Instead, sulfur seems to have a stimulating effect upon the growth of live tree stumps.

Common rock salt, when applied to oak stumps which have been allowed to grow for several years, does not do much harm when applications of from 2 to 2½ tons per acre are given. When applied in amounts of ½ to 1 ton per acre, these shrubs are stimulated, the salt acting as a fertilizer.

In cases where trees have been cut in winter time and the brush burned at the place, the stumps are much retarded in their growth by applications of salt at the rate of ½ to 1½ tons per acre. If applications of 2 to 3 tons per acre are made, stumps of white oak and black oak are killed or so severely injured that death results.

The best time to make salt applications is in the spring, just before the leaves appear and when the sap stream is able to carry the chlorine to the buds and young leaves.

PLATE 1

FIG. 1. Cleared land in early spring when the applications were made. In the foreground, scattered broadcast; in the background, application on and around the stumps.

FIG. 2. Same field in September. The application was approximately 2 tons of common rock salt per acre, on and around the stumps.

FIG 1

FIG 2

PLATE 2

FIG. 1 Plot which received approximately 2 tons of salt per acre, scattered broadcast.
FIG. 2 Check plots

Fig. 1

Fig. 2

A NEW SOIL ELUTRIATOR

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INTRODUCTORY

Briggs (1, 2) pointed out many years ago that the geometrical configuration in a soil consists of tortuous liquid-solid, liquid-gas, and gas-solid surfaces intersecting and meandering in devious ways. Such configuration, usually and perhaps never at equilibrium, may be expected, from the purely mechanical point of view, to be controlled primarily by the effective size of the particle, the coefficient of viscosity (η) of the liquid and the surface tension (T) of the liquid-air surface, although, as Briggs states, the resultant at the curve of intersection involves two additional vectors. The pressure of a liquid under a curved surface involves the surface tension (T) and the curvature of the surface, the latter depending primarily upon the "degree of dispersion"[1] of the solid phase, or the effective radius of the soil particle. Colloidal substances move with the liquid and may temporarily be ignored, except in so far as they may affect the viscous constant. It is apparent, therefore, that a complete general solution of such problems as the movement of capillary moisture in a soil may be available, involving such parameters as may be used to define the "dispersion" of the solid phase, i.e, the mean, the mode, and the average deviation of the grams-radius distribution function, the surface tension (T), and the curvature of the liquid-air surface, the latter involving the "dispersion" parameters as well as the two other surface tension constants.

The author (3) has proposed a tentative general solution of the problem of capillary flow, following in part the solution of Slichter (4) for underground water. Slichter's solution, however, does not involve a liquid-air surface. A capillary transmission constant has been introduced which involves implicitly the parameters above named, the ratio $\left(\frac{T}{\eta}\right)$ affecting this constant as a linear factor. A laboratory investigation is under way at the Utah Agricultural Experiment Station with the view of investigating the influence of this ratio, and additional plans are being made for investigating the influence of the "dispersion" parameters. It was found, however, that neither suitable soil separates nor a method of obtaining them in quantity were available and it is the primary purpose of this article to describe what promises to be a successful piece of apparatus for obtaining appropriate soil separates, though involving little that is essentially new in principle.

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[1] This term is borrowed from Colloidal Chemistry and perhaps not incorrectly applied to the rigid soil particles.

DESCRIPTION OF ELUTRIATOR

The diagrammatic sketch of figure 1 will convey at once information as to the nature of the process involved. A steady stream of muddy water is introduced at the bottom of the smallest of a series of 19 cylindrical vessels ranging in diameter in arithmetic progression from 4 to 40 cm. The stream leaves the first cylinder through a conical cap, entering the second through a conical base, and so on throughout the series.

The apparatus was constructed for our laboratory by the Central Scientific Company, the cylinders being made of brass with glass windows and detachable cap and base. Small glass cylinders were subsequently attached at the base of each cylinder through perforated rubber stoppers to serve as storage reservoirs for the particles which, contrary to original expectation, were found constantly descending into the narrow part of the conical base. In order to provide an escape for air accidentally entering the apparatus and in order that the samples collected from the tubes might be rewashed, the top of each cylinder was made to open into a vertical glass tube terminating with a funnel. Each sample can thus be readily drained off through a rubber tube attached at the lower end of the reservoir and introduced into the funnel next preceding the cylinder from which it was taken.

A stream of clear water kept constant by a simple constant head device is introduced into an appropriate vessel (*A*, fig. 1) into which the heterogeneous soil is periodically introduced and kept agitated by an electrically driven stirrer. The muddy water overflows into funnel no. 1, entering the elutriator as stated. In rewashing any of the several separates the stirring apparatus may be readily washed by draining through a tube at the bottom and the separate introduced in place of the heterogeneous soil, overflowing into the funnel immediately preceding the cylinder from which it was taken.

It is apparent that the absolute diameter of the tubes with a given current fixes the size of the particles and the size of the current fixes the rate at which soil may be handled. For the case described below a current of approximately 0.7 liter per minute was used. The velocity of the constant stream varies inversely as the square of the diameter of the cylinder,

$$v_1 = \frac{K^1}{d_1^2}$$

whereas the relative velocity of the falling particle, according to Stokes' law, is proportional to the square of the diameter of the particle,

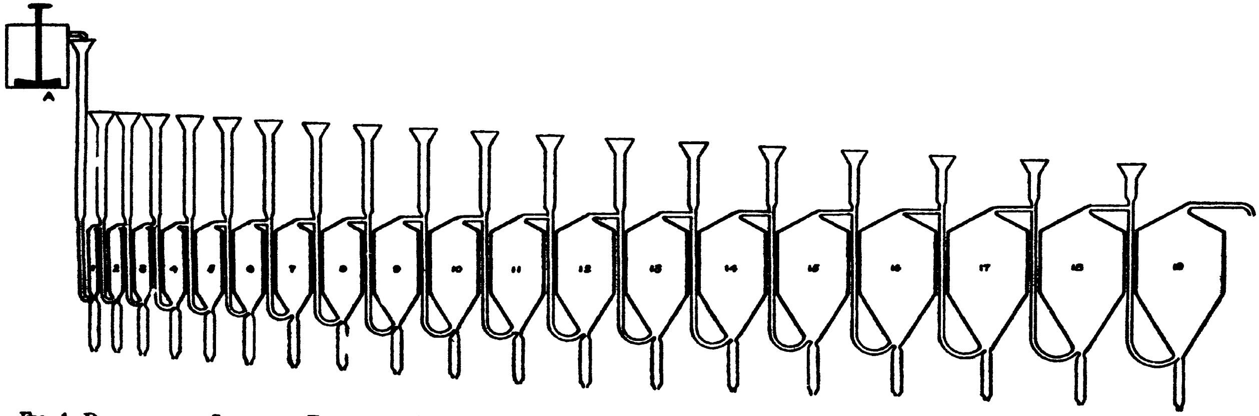

FIG. 1. DIAGRAMMATIC SKETCH OF ELUTRIATOR SHOWING COMPLETE SERIES OF NINETEEN UNITS, TOGETHER WITH FEEDING AND STIRRING DEVICE SHOWN AT *A*

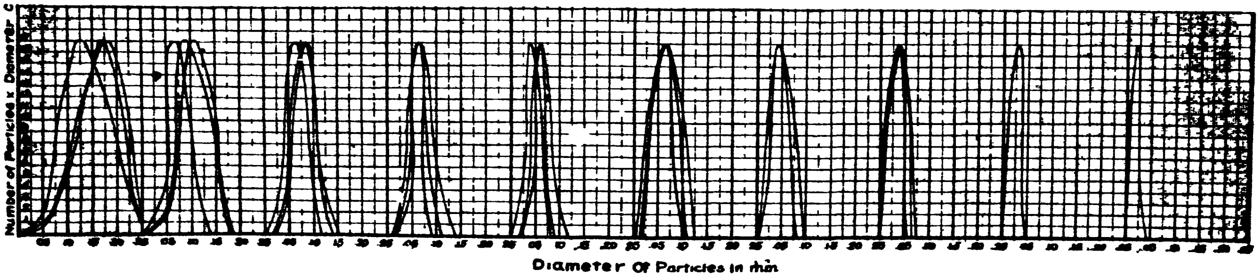

FIG. 2. PLOT OF THE GRAMS-DIAMETER DISTRIBUTION FUNCTION FROM ACTUAL COUNT AND MEASUREMENT WITH THE AID OF THE MICROSCOPE
Samples analyzed taken from units 1–7, 9, 14, 19

$$v_2 = k_2 d_2^2.$$

When the velocity of the water, v_1, is equal to the velocity of the particle, v_2,

$$\frac{k_1}{d_1^2} = k_2 d_2^2$$

and

$$d_1^2 d_2^2 = k_3$$

where d_1 and d_2 are the diameters of the cylinder and soil particles, respectively. Since, therefore, the diameters of the cylinders increase in arithmetic progression it is apparent that the diameters of the particles will diminish in harmonic progression.

The diagram of figure 2 represents the plot of the grams-radius distribution function or mechanical analysis made by actual count with the aid of the microscope of the soil from each tube of the series after a first, second, and third elutriation. The soil used contained very little organic matter and a large part of the clay had been previously washed out, so that with the mechanical stirring device described the aggregates were readily disintegrated before the muddy stream was introduced into the apparatus. The ordinate represents the product of the number of particles of the respective diameters and the cube of the diameters, the abscissae representing a range of 0 to 0.25 mm. For each tube the distribution function was determined approximately for each of the three elutriations, and a slight shift of the curve is observed in most cases after each washing, as well as a decided narrowing of the area under the curve. Under observation with the microscope a decided improvement was noticed after the second washing, as may also be seen from the microphotographs shown in plate 1, these photographs being arranged horizontally in order of changing size and vertically in order of elutriation. Since, however, the cube of the diameter was plotted as ordinate in order to harmonize with the usual method of making a mechanical analysis, the change is less noticeable on the graph. Several of the units after no. 7 were missing, but the modes of the respective curves were found to fall approximately in their proper places in the harmonic series one-fourth, one-sixth, one-eighth, etc., lending incidental support to the application of Stokes' law in this case.

The apparatus has given excellent satisfaction for our purpose, although as stated several units of the series after the seventh are missing from the set now in use. It is only accidental that a choice of diameters increasing in arithmetic progression was made, but in view of the fact that the influence of the diameter of the particle on the physical properties of the soil becomes increasingly important as the diameter diminishes, it is possible that the harmonic ratio may prove entirely appropriate.

In conclusion, acknowledgment should be made to Mr. N. E. Edlefsen and Mr. Scott Ewing for able assistance and valuable suggestions that have been offered, and to Dr. F. L. West, head of the Department of Physics, for coöperative encouragement during the progress of the work.

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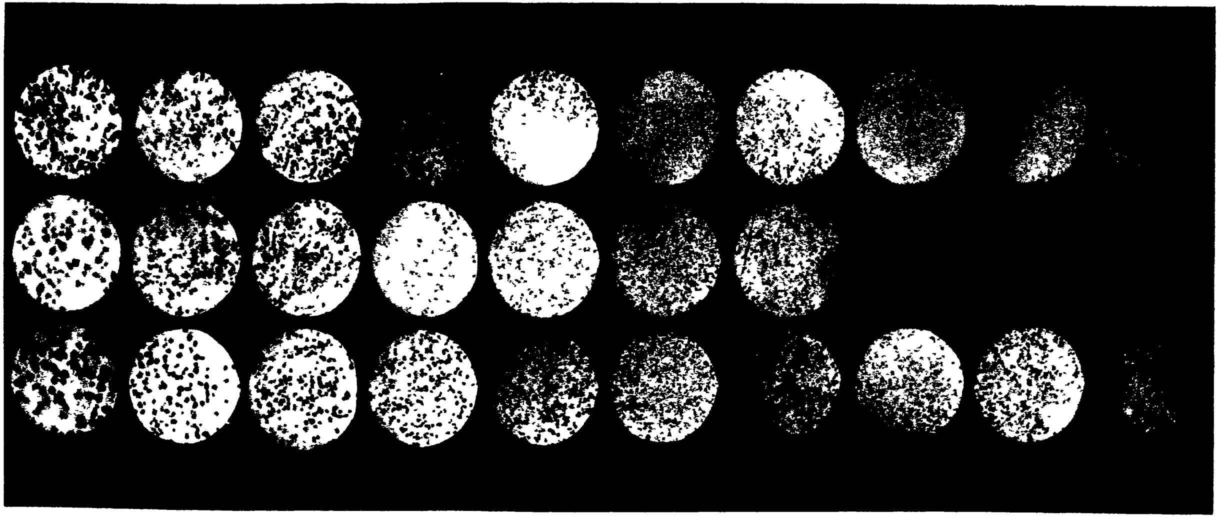

PLATE 1

MICROPHOTOGRAPHS OF SOIL PARTICLES FROM THE RESPECTIVE UNITS OF THE ELUTRIATOR AFTER A FIRST, SECOND, AND THIRD ELUTRIATION

The photographs are arranged horizontally in order of diminishing size of particle and vertically in order of elutriation. Photographs were not available for the finest particles after the second elutriation

REDUCTION POTENTIALS OF BACTERIAL CULTURES AND OF WATER-LOGGED SOILS

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The importance of oxidational processes in soils is attested by a voluminous literature, and has been emphasized and studied from various angles in publications from this laboratory by Oswald Schreiner and collaborators (31, 33, 34, 36, 37). A review of the literature and a broad discussion of the subject have been given in these publications (especially 31, 33). By the use of chemical tests it has been found that soils are often oxidizing, and it has been concluded that, as a rule, good "vital" soils are more so than poor soils. It has also been suggested that it is possible for a soil to be too oxidizing (34), though there is not yet a satisfactory way of determining by laboratory examination when such a condition exists. There is also a considerable literature on oxidation and reduction by yeasts and bacteria, which has not yielded the fundamental results which may be eventually expected. Reduction processes in soil have also been studied, though to a lesser extent (18, 36).

Just as a sharp line can be drawn between acidity and alkalinity, taking the reaction of pure water as the standard, so can a sharp line be drawn theoretically between oxidation and reduction, taking as a standard the properties of pure water (39). The parallelism goes a long way. Both acidity and oxidation are relative terms and such absolute theoretical distinctions are of limited significance. A neutral condition is not necessarily the optimal condition for soils, in fact is not acid enough for certain systems of cropping (13), and the theoretical point between oxidation and reduction is probably not the best condition for soils. Differences in acid intensity have been studied of late in soils by the use of an electrode saturated with hydrogen gas at the atmospheric pressure. Let us consider differences in intensity of oxidation and reduction, as studied by means of an electrode of noble metal, upon which no pressure of hydrogen or oxygen is purposely applied.

Energy in its various forms has two factors, intensity and quantity. This is true not only of energy in the usual physical sense, but also in a very broad sense, including the energy of acidity (of which the two factors may be taken as hydrogen-ion exponent[1] and quantity of acid) and the energy of oxidation and reduction. In various studies on oxidation and reduction in soils only the quantity factor has been studied, such as the oxygen consumed or the carbon dioxide given off, or the quantity of colored substance produced by the action of soil on a given suitable reagent. Other things being equal, the intensity factor might be assumed to vary in parallel with the quantity factor, but unfortunately this assumption is very doubtful when different soils are being compared.[2]

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[1] Dr. E. Q. Adams pointed out to the writer that the hydrogen-ion exponent, rather than the concentration, is the true intensity factor of acidic energy, in properly chosen units.

In the case of oxidation and reduction, the so-called oxidation or reduction potential represents the intensity factor. This potential is the electrical potential difference between a solution and a chemically indifferent electrode immersed in it. If two such electrodes are set up, immersed in two liquids of different oxidation potential, the liquids being connected by a siphon, then on making metallic electrical connection between the electrodes a current will pass, and oxidation of the solution about one electrode will occur and reduction of the solution about the other.[3] Which solution is oxidized at the expense of the other will depend upon which oxidation potential is the greater, and if the two potentials happen to be the same no current will pass and no chemical action take place. In general, the magnitude of the potential difference between the two electrodes gives no information as to the quantity of current which can be obtained from the system, and in many cases very little current can be taken without lowering excessively the potential difference. It will be observed that oxidation and reduction potentials always occur in pairs, there being apparently no certain way of obtaining a single potential with any accuracy. However, cells may be set up in which there is only one variable half-cell, in which case we may treat the total electromotive force as the oxidation or reduction potential, taking for purposes of comparison the constant half-cell as a zero. A calomel electrode is generally taken as the constant half-cell, and its potential is sometimes taken as zero, sometimes as some other more or less arbitrary number. By referring the oxidation potential of a variety of oxidizing and reducing agents to a calomel electrode for comparison, Neumann (23) was able to arrange the various agents in a continuous series, using largely the measurements given by Bancroft (2). It is well known that this cannot generally be done by chemical means, and it unfortunately follows that such electrical measurements do not always predict successfully the chemical behavior of two given solutions when they are *mixed*. The results may of course depend on reactions of different type, especially upon catalytic actions. The significance of the electric potential in chemical reactions is discussed at length in Chapters XIV and XV of Stieglitz's *Qualitative Analysis* (35).

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[2] Suppose a certain soil is found to absorb oxygen at a higher rate than another soil, and so may be properly said to respire with greater intensity; this fact does not show whether the soil contains very easily oxidizable bodies, for it may contain instead a large quantity of less easily oxidizable bodies. A study of the intensity factor would be necessary for such a distinction

[3] It is to be understood that oxidation and reduction are taken here in the broad sense, referring either to the participation of oxygen or hydrogen or to a mere change of valence.

By comparing the oxidation potential of oxygen in solutions of various alkalinity with that of various mixtures of potassium ferro- and ferricyanide solutions (the potentials of which are practically unaffected by changes of reaction if substantial quantities of both forms are present), Fredenhagen (11) was able to show that there was a particular ratio of ferro- to ferricyanide which was stable at a definite alkalinity. At greater hydrogen-ion concentrations the proportion of ferricyanide in the mixture would be greater, for equilibrium with oxygen. In the soil, the oxidizing potential of oxygen (at a pressure of 0.2 atmosphere) would also be greater under acid conditions, and oxidations in acid soil should therefore on the whole go further than in neutral or alkaline soil. In bacterial cultures or in water-logged soil the hydrogen-ion concentration establishes a limit of reduction potential (the potential of the hydrogen electrode gives this limit), which cannot be greatly exceeded without the liberation of hydrogen gas.[4]

It is obvious that measurements of hydroxyl-ion or hydrogen-ion concentration are necessary for the fullest interpretation of oxidation or reduction potentials. Wells (39) and Frary and Nietz (9) have recently made use of such hydrogen-ion concentration data in the interpretation of oxidation potentials. It is doubtful, however, whether all interpretation of oxidation potential must be made in this way. The Nernst theory, that oxidation and reduction potentials are due to charges of oxygen or hydrogen gas (or of both, in equilibrium), has been vigorously disputed by van Laar (38), who says that in many cases such gas charges are fictitious. The fundamental thing which determines the occurrence of oxidational reactions in electric cells is not the pressure of oxygen or hydrogen gas, but is the actual potential itself, and the writer has therefore preferred not to "correct" such potentials by means of the hydrogen-electrode potentials, except in special cases, where an equilibrium involving gaseous hydrogen seemed to exist.

TECHNICAL DISCUSSION

Solutions differ in their action on an indifferent electrode; in some, like mixtures of potassium ferro- and ferricyanides, the electrode exhibits quickly a constant and reproducible potential (20), whereas in others, such as photographic developers (9) the potential varies for weeks. In the latter case it may often be difficult to show that the potential measurements have much significance. If the potential is the result of an equilibrium between one substance and its immediate oxidation or reduction product, and both substances are present in fair concentrations, then, as Peters showed for various salts of iron (26), constant and reproducible potentials are exhibited, the potential can be calculated by van't Hoff's equation from the concentrations of the substances involved, and the potentials have therefore a definite chemical meaning. In cases where the potentials are inconstant, even if they are simply keeping pace with a progressive chemical reaction, it is difficult to establish the chemical significance. The tendency of a chemist is therefore to avoid the study of systems which do not give constant potentials, but a progressive variation of potential in a biological system such as soil should not deter the investigator, because biological significance may be capable of demonstration, even if chemical significance may not.

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[4] Unless the phenomenon of overvoltage is possible in these cases. No such phenomenon was met in this work. It might perhaps be expected in media rich in sulfur.

The definiteness of the intensity factor in oxidation is dependent to some extent upon the magnitude of the quantity factor. The situation is similar with regard to acidic energy. The behavior of a hydrogen electrode is unsatisfactory in solutions having minimal "buffer action," and we may speak of the ferro-ferricyanide mixtures as being well provided with *oxidative buffer* properties. The usual term "buffer action" may be distinguished when necessary by the qualification *reaction buffer*. Soils, as well as bacterial cultures, show little oxidative buffer as compared with ferro-ferricyanide mixtures, and this is the cause of a fundamental difficulty in soil work. Because of this it is necessary to add water to the soil in which an electrode is immersed in order both to secure good electric contact between the soil and the electrode and also to exclude air, for the oxygen of the air goes into quick reaction with the electrode, disturbing the potential and making it uninterpretable. It is therefore somewhat difficult in such cases to assume any significance to potentials unless they are definitely different from the potentials which atmospheric oxygen would impart, because if oxygen values are seen, it may be objected that they are due merely to oxygen carried on the surface of the electrode or dissolved in the soil, the soil itself being inert. It is because of this difficulty that the soil work reported in this paper is confined to water-logged soils. It may, however, be perfectly possible in some cases to obtain significant potentials a short time after wetting the soil and introducing the electrode.

All the "potentials" recorded are the electromotive forces of the entire cell, including the saturated potassium chloride calomel electrode. In most cases the positive terminal of the cell was the mercury of the calomel electrode (as is the case in ordinary hydrogen electrode measurements) and in these cases the potential was recorded positive, so that high positive values represent a high intensity of reduction, and high negative values would represent high intensities of oxidation. The sign, therefore, refers to the solution in all cases, and not to the electrode immersed in it (which of course has the opposite sign). It was until lately the practice to correct the observed potential differences for an assumed value for the single potential of the calomel half-cell, taking the 1 N KCl calomel electrode potential equal to 0.56 volts, the mercury being positive to the potassium chloride solution. This assumption is equivalent within 0.01 volt to taking the 0.1 N KCl calomel electrode potential as equal to 0.62, and the saturated KCl calomel used for these measurements as equal to 0.53, at the ordinary temperatures (20–30°C.). In these cases also the mercury is positive. If therefore we correct our observed potentials by subtracting 0.53, the results will be comparable with the table of "absolute potentials" of Neumann (23, p. 228), and of van Laar (38), in which the single potentials refer to the potential of the *solution* against the electrode immersed in it; but if we wish to compare them with the absolute potentials given by Wells (39), we must subtract 0.53 and then change the sign of the result, for in his table the single potentials refer to the potential of the *electrode* against the solution. The potential of the normal hydrogen electrode is of late taken as an arbitrary zero, which gives 0.283 instead of 0.56 for the 1 *N* electrode, and therefore 0.25 instead of 0.53 for the saturated electrode used. At present it seems well to present the potentials uncorrected. If, during any measurement, the saturated electrode had been removed and a 1 *N* electrode substituted, the fluid or soil would have appeared to be about 0.03 volt more reducing.

All of the experiments except the last soil experiment were done in 1915, and the potentials were then measured by means of the voltmeter scheme given by Hildebrand (15) with the use of a capillary electrometer. The voltmeter readings were 1.5 per cent too high (after the zero position of the needle was adjusted) and this correction (determined at the time with the kind cooperation of Dr. W. Mansfield Clark) was applied. For the other experiment a good potentiometer was used with a galvanometer, the coil of which has a resistance of about 400 ohms.[5]

Various metals have been used for the unattackable electrode. Platinum has been used either blank, or made gray (2) by coating with platinum black and heating in the flame. Mercury has been used, for solutions not so oxidizing as to act on the mercury, producing a calomel electrode (7, 8). Gold has been especially recommended by Lewis and Sargent (20); gold electrodes were found sensitive to light by Bose and Kochan (3), a matter of 0.1 volt. The writer has not used gold, and has not seen any certain light effects; light, however, was excluded to a reasonable degree in all the measurements reported.

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[5] A voltmeter reading directly to 20 millivolts (0.020 volts) is fairly satisfactory even for much hydrogen electrode work, if calibrated, and if tenths of a division can be accurately estimated. The homemade capillary electrometer used (Ostwald form) was more satisfactory than the voltmeter. Not too fine capillary tubing was used. It was sensitive to about 0.0005 volt, applied across the terminals, and in series with a gas chain of high resistance it was still possible to read to 0.001 volt; whereas a good galvanometer, unless its coil has a high resistance (say 400 ohms), may fall far behind a capillary electrometer if used in a high-resistance circuit. It is necessary to maintain the short-circuit (except at the moment of use) through secure connections (platinum contacts, well-soldered connections, or freshly-applied pressure by screws). It was seldom necessary to blow over a drop of mercury, as care was taken not to hold down the key long enough to overcharge the electrometer. The surface of the mercury can be put in good condition, when necessary, by applying an electromotive force about 0.8 volt, connecting the capillary pole with the negative lead (so that the mercury contracts in the capillary) and then letting stand 15 to 30 minutes short-circuited (25). Simple electrical shielding (40) was found to prevent the trouble otherwise experienced with the capillary electrometer outfit in damp weather.

REDUCTION POTENTIALS OF MICROBIAL CULTURES

Potter (27, 28) measured the electromotive force produced when two platinum electrodes were placed in two portions of culture medium, separated by a porous partition, and one portion was inoculated with yeast or *Bacillus coli*. In both cases the electrode in the inoculated portion was more zincative (negative) than the uninoculated portion. Glucose medium was used for yeast, and a synthetic medium containing ammonium tartrate, asparagin, and mineral salts for *B. coli*, also for the latter organism a medium with starch substituted for the asparagin. The maximum electromotive forces developed were 0.3 to 0.4 volt, and in the last case 0.5 volt. He did not interpret these results in terms of oxidation or reduction, in fact stated that no electric effects could be produced by oxidation of the platinum electrodes, but spoke of the liberation of electrons of the organic substance during the decomposition and of the electrical charges set free in the vital processes of the microorganisms. The difference of interpretation, however, is chiefly a matter of language alone. The direction of the electromotive forces described is such as to point to the establishment of reducing conditions by the microbial activity. No potentials against a calomel electrode were measured. Varying the concentration of yeast, Potter found the maximal electromotive force to be developed more quickly, the more concentrated the yeast suspension.

Mixed culture, mercury electrode

A considerable number of experiments were made by the writer using a mercury electrode, of which only a few will be given. The electrode vessel was the glass part of an ordinary spirit lamp. By means of appropriate glass connections the electrode vessel could be completely filled with mercury, displacing all air in the vessel and in the tube used for filling. This tube was long enough to reach to the bottom of the cultures examined. Stopcocks made it possible to make a liquid contact with saturated potassium chloride solution leading to the calomel electrode, and by admitting more mercury an old contact could be forced out and a new one made at any time. The vessel could be gently shaken to aid the attainment of equilibrium. This arrangement was used to examine bacterial cultures, and gave good results in the examination of mixed cultures grown in deep layers when care was taken to avoid the entrance of air. Such cultures were carefully protected against agitation. Though no test was made, the cultures undoubtedly contained anaerobes. If air was purposely or accidentally admitted to the cultures, the potentials were disturbed and sometimes a prohibitive length of time was required to re-establish equilibrium. Figure 1 shows the time curves for a mixed culture in a deep layer of 1 per cent Witte peptone. This culture was obtained by inoculation with soil one month previously, and had stood a number of weeks without agitation before it was introduced into the electrode vessel. In figure 1, curve *A* shows the change of potential with time when the culture was introduced with care to avoid entrance of air, curve *B* shows the relation for the same culture when a little air accidentally gained access, and curve *C* shows the relation when the culture was shaken with air. By admitting air in small quantity to the culture in the electrode vessel it was possible to disturb the potential in less degree, and in this case the potential was observed to return to the former value in a few hours. Attempts to displace the equilibrium in the opposite direction by passing in hydrogen gas failed. The culture was nearly neutral to litmus paper.

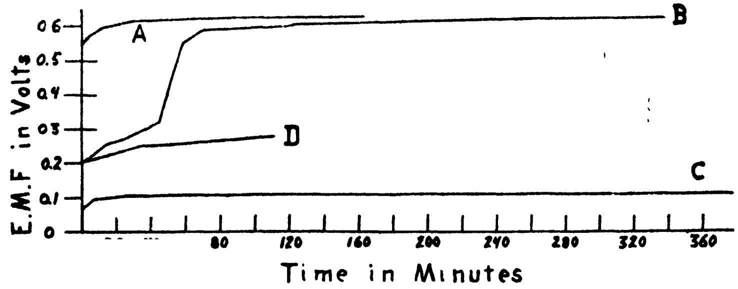

Fig. 1. Reduction Potentials of Bacterial Cultures

A similar culture 17 days old was tested in the same apparatus with careful exclusion of air and in addition the hydrogen electrode potential was determined. Constant potentials were found, 0.630 and 0.707, for the mercury and hydrogen electrodes, respectively.

A similar culture 20 days old, grown in a different vessel, gave in the same apparatus the following constant potentials for the mercury and hydrogen electrode potentials, respectively: 0.616 and 0.715.

In these cases a little time was required for the reduction potential to become constant, as in the case of the experiment plotted in figure 1. The final values for the reduction potential were in fair agreement, being 0.620, 0.630, and 0.616, under conditions not exactly the same.

Bacillus subtilis, amalgam electrode

A sheet of platinum about 1 by 3 inches, provided with a platinum wire connection sealed through a glass joint, was rolled into a hollow cylinder and amalgamated by immersion in boiling mercury. It was introduced by means of the glass joint into a test-tube, to the bottom of which was sealed a T-form double stopcock. One arm of the stopcock was used as a pipette for filling and the other for making liquid contact with saturated potassium chloride solution (which was connected with the calomel electrode). An old culture (one month) of *Bacillus subtilis* on 1 per cent Witte peptone, the growth of which had sunk to the bottom, was introduced to the electrode vessel, completely filling it to the exclusion of air. The observed potential was 0.19, rising to 0.26 in 30 minutes. By means of an auxiliary electrode (replacing the calomel electrode) the reduction electrode was polarized until its potential (measured against the original unpolarized calomel electrode) was 0.314. On standing, the potential returned in an hour to the value 0.23, showing that this value represented an equilibrium, if only a temporary one. The hydrogen electrode potential was 0.753.

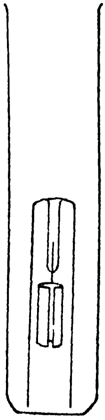

FIG. 2 "INVERTED-VIAL ELECTRODE"

Another experiment was performed with the same amalgamated electrode. A 3-day culture of *B. subtilis* was used, the culture having been grown on a mixture of Witte peptone 1 per cent, dextrose 1, and K_2HPO_4 0.5 per cent. The reduction potentials are shown in figure 1, curve *D*. The highest reached was 0.275; the hydrogen-electrode potential was found to be 0.622.

Bacillus coli in inverted vial electrode

B. coli was grown in an "inverted vial" provided with a bare platinum electrode, as shown in figure 2. After the growth, mercury could be introduced into the tube carrying the platinum wire, for metallic connection with a copper wire leading to the voltmeter arrangement. Two cultures of *B. coli* were obtained through the kindness of Dr. W. M. Clark. They had been found to be typical cultures, with a gas-ratio, $CO_2:H_2$, nearly equal to 1, as determined by exact analysis over mercury (29). The reduction potential was measured before the bubble of gas touched any part of the platinum. The sheet of platinum was about 1 inch square. The culture medium was Witte peptone 1 per cent, dextrose 1, and K_2HPO_4 0.5 per cent. Liquid contact was made by means of a capillary tube filled with saturated potassium chloride solution, which was dipped to the bottom of the tube with as little agitation as possible. The temperature was about 25°C. The hydrogen electrode potentials of both cultures were also measured. All potentials, reduction and hydrogen, appeared quite constant. From the reduction and the hydrogen potentials the pressure of gaseous hydrogen was calculated from the equation: $\log pH_2 = -33.8E$, where pH_2 is the pressure in atmospheres of gaseous hydrogen, E is the difference in volts between the hydrogen and reduction electrode potentials, and 33.8 is the reciprocal of the familiar factor $RT/2F$ after correction for conversion of logarithms.[6] The results are shown in table 1.

TABLE 1

Reduction and hydrogen electrode potentials of two cultures of B. coli, and the pressures of gaseous hydrogen calculated therefrom

CULTURE	OP	BX
Reduction potential (volts)	0.580	0.516
Hydrogen potential (volts)	0.594	0.554
E (volts)	0.014	0.038
Value of $\log pH_2$	−0.473	−1.282
$\log pH_2$	1.527	$\bar{2}.718$
Pressure of hydrogen gas (pH_2) (atmospheres)	0.34	0.052

It is not supposed that such gas analysis is accurate, but the fact is of interest that the potential of bare platinum pointed to concentrations of hydrogen gas of the order of magnitude that would be expected. For the ratio, $CO_2 : H_2$, equal to 1, the pressure of hydrogen would be 0.5 atmosphere, calling for a difference of about 0.009 volt between the reduction and hydrogen-electrode potentials. From table 1 it is seen that the calculated pressures were 0.34 and 0.052. These values are less than that established by exact analysis of the gas obtained by growing the organism *in vacuo*, but if oxygen is not excluded the analysis also shows smaller proportions of hydrogen gas (17).

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[6] This equation represents the relation between two hydrogen electrodes at different hydrogen gas pressures (one atmospheric pressure, and the other, to be determined), immersed in a solution of one hydrogen-ion concentration, and can be obtained, for instance, from an equation given by Wells (39), second equation on page 58, by applying this equation for the single potential to the case where the pressure of hydrogen gas is 1, when the log term becomes 0, and then to the case where the pressure is pH_2, and finally subtracting one result from the other. This equation then applies not only to a neutral solution but to any other, since the equation from which it was derived is affected by changes in reaction only in the term −0.136 (the minus sign was omitted in the bulletin through a printer's error), which disappears in the subtraction, whatever its value may be at the particular hydrogen-ion concentration.

Reduction potentials of bacterial suspensions

Thick suspensions of bacteria produce reduction of methylene blue far more quickly than cultures, or thin suspensions. Such suspensions have been used in studies of bacterial reduction of colored substances (5, 14, 24).[7] *B. coli* "hx" was suspended in 0.9 per cent sodium chloride solution with the addition of a little broth, and measured in an apparatus similar to the amalgamated electrode vessel, but the electrode itself was bare platinum, not amalgamated. The temperature was 26°C. The progress of the experiment can be seen from figure 3. The maximal reduction potential was 0.599 volts, decreasing to 0.594, doubtless because of acid formation, as the hydrogen electrode potential, subsequently determined, was 594, decreasing to 592. Thus the two potentials were very nearly the same. *Bacillus mycoides* was grown on agar and suspended in broth. It was studied in the amalgam electrode, and the results are shown in figure 3. The highest reduction po-

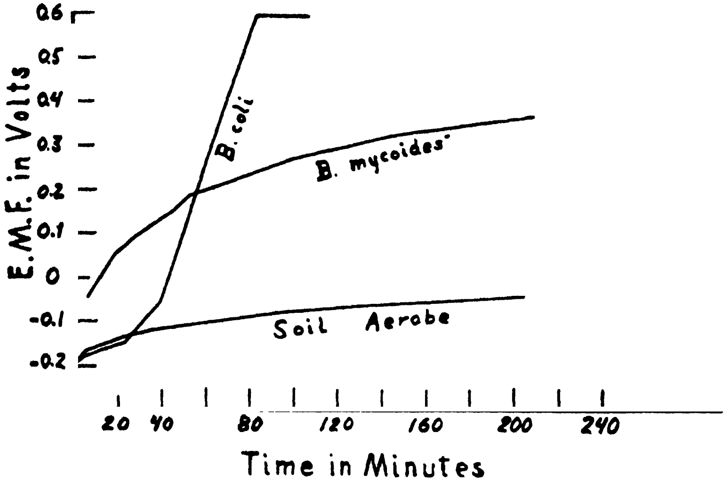

FIG. 3. REDUCTION POTENTIALS OF BACTERIAL SUSPENSIONS

tential was 0.37; whereas the hydrogen electrode potential was 0.700. An unidentified soil aerobe, motile, liquifier of gelatine, not identical with *B. mycoides* or *B. subtilis*, was grown on agar, the growth suspended in a mixture of equal parts of 0.1 *M* Na_2HPO_4 and 0.1 *M* NaH_2PO_4, and examined with a gray platinum electrode. In this, as in all these cases, the electrode vessel was of course completely filled with the suspension, to the exclusion of air. The highest reduction potential was −0.04, whereas the hydrogen-electrode potential was 0.652. The intensity of reduction was therefore very low in comparison with the other cultures, but this culture gave a good indophenol test with Ehrlich's reduction reagent (6), a mixture of para-nitrosodimethyl anilin and alpha-naphthol.

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[7] To the data of Harden and Zilva may be added the following facts, determined by the writer in 1910 but not published. When masses of *B. coli* were washed so that on the addition of methylene blue solution alone no reduction could be seen on incubation at 37°, and the effect of various substances on the reduction was examined, dextrose and glycerol were found to be active in especially small concentrations. At least as small concentrations as 0.02 and 0.002 per cent, respectively, caused the reduction of the methylene blue to take place. Traces of ammonium hydroxide were also very active, and this seemed to make tests of the activity of the sodium salts of weak organic acids uninterpretable without hydrogen-ion control. Dextrose and glycerol were found by Cole (5) to be very effective also in aiding the formation of methemoglobin from hemoglobin by the action of pneumococcus suspensions.

REDUCTION POTENTIALS OF WATER-LOGGED SOILS

Hagerstown loam

Two hundred grams of soil, stated to be Hagerstown loam, were placed in a tumbler and enough water added to saturate the soil and make a thin layer of water over the top. Similar tumblers were prepared, using the same soil to which dextrose had been added before wetting. Gray platinum electrodes about 1 inch square, attached to platinum wires sealed into glass, were introduced by opening the soil with a knife; the soil was pressed back, and the whole allowed to stand. At daily intervals, liquid contact with the saturated potassium chloride solution was made by laying a capillary tube filled with the potassium chloride solution on the soil, and the potential against the calomel electrode was measured. After each measurement the capillary tube was removed, and the soil which was contaminated with potassium chloride was scraped out. The reduction potentials are given in table 2.

TABLE 2

Reduction-potential experiment with Hagerstown loam plus dextrose

DEXTROSE		REDUCTION POTENTIALS AFTER VARIOUS PERIODS			
		1 day	2 days	3 days	4 days
gm.	*per cent*				
0	0	−0.32	−0.16	+0.07	+0.11
0.25	0.125	−0.18	+0.44	0.32	0.26
0.50	0.250	−0.24	+0.44	0.31	0.31
1.00	0.500	−0.32	+0.46	0.48	0.45
2.00	1.000	−0.02	+0.48	0.52	0.50

Some of the irregularities may be due to the fact that the layer of water evaporated, leaving in some cases fine cracks which permitted air to gain entrance to the layers of soil near the electrode. The layer was restored by daily addition of water. It is noteworthy that the smallest quantity of dextrose added to the soil, about 0.1 per cent, produced a very large effect on the soil after water-logging for two days.

Duplicate tumblers were prepared and young rice seedlings were replanted in the water-logged soil, the soil being kept just wet. In 2 days, differences could be seen, and in 3 days the rice seedlings were apparently normal in the water-logged soil containing no dextrose, but were progressively poorer in the soils to which dextrose had been added. The injurious effect was definite with only 0.125 per cent of dextrose, and the plants were in very bad condition with the higher concentrations. The experiment with rice is only suggestive, as a number of factors were not studied. It was not known at the time how to measure the hydrogen-ion concentration of soils.

A less extensive experiment showed that Witte peptone added to the same soil produced reducing conditions, when the soil was water-logged, but the effect was not so marked as in the case of dextrose.

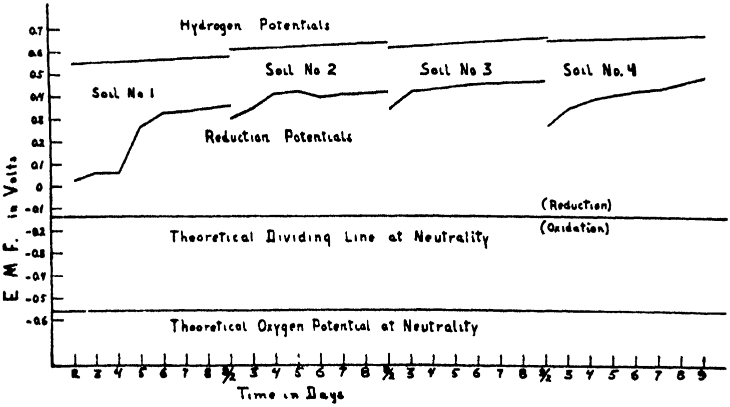

FIG. 4. REDUCTION POTENTIALS OF WATER-LOGGED SOILS AT VARIOUS TIMES

Various soils without dextrose

As electrode vessel, a tube was used open at both ends. A platinum electrode coated with palladium black, sealed into a glass tube, was carried on a rubber stopper fitting into the tube. The electrodes were much smaller than those used previously, being only 3 or 4 mm. square, and were treated as for a measurement of hydrogen-ion concentration, but were well exposed to moist air before use, in order to permit the removal of hydrogen which had been deposited in the sulfuric acid "cleaning" bath. The tube was filled as tightly as possible with wet soil, using as thick a mud as could be handled without inclusion of air. After filling the tube, the end was closed with thin rubber sheeting, secured with rubber bands. Each day the rubber sheet was removed, contact made with a rubber tube containing saturated potassium chloride solution, and the potential measured. After the measurement the contaminated soil was at once scraped out and replaced with fresh soil, without disturbing the layers of soil about the electrode, and the rubber sheet replaced.

Four soils were studied. No. 1 was Caribou loam; no. 4, Washburn loam, the others being soils from another region. In figure 4, the reduction potentials thus measured are shown, as curved lines. The straight, slanted lines show the hydrogen electrode potentials for the soils, determined without water-logging, and at the end of the period of water-logging, in each case. For purposes of illustration, the theoretical line dividing oxidizing from reducing solutions at the hydrogen-ion exponent 7, has been shown, as well as the potential which an oxygen electrode would show (19) at the same hydrogen-ion exponent if the experiment could be correctly performed. The hydrogen electrode potential line for the exponent 7 has not been drawn, since it is so near the slanting lines as to produce confusion.

There appears to be a significant difference between the Caribou loam (no. 1) and the other soils, as water-logging produces a much slower effect with it than with the others. To make sure of this point the whole experiment was repeated, using the same electrodes as before, but without recoating, or even cleaning beyond simple rinsing, and the electrodes were interchanged, so that no soil was tested with the same electrode again. The results were very similar, the main difference being that the rate of production of reducing conditions was different, presumably because of a change of room temperature.

SOURNESS OF SOILS VS. ACIDITY OF SOILS

A few years ago an anomalous situation existed with regard to soil acidity. It was held by many that the reaction of ordinary soils was either neutral or slightly alkaline, reactions with blue litmus paper notwithstanding. The reddening effect of certain soils upon blue litmus paper was held to be due solely to adsorption. At the same time many investigators were applying litmus paper to detect soil acidity and recommending its use to the farmer, holding to the point of view that although soil acidity may not be proved by the use of litmus paper, the reddening effect on blue litmus paper is often correlated with the failure of red clover and other crops supposedly intolerant of acid conditions. By such investigators the word "acidity" was applied to soil problems with due regard to the opinions then current and quotation marks about the words "soil acidity" and various qualifying phrases were often used in order to waive the question as to whether a "true" acidity was involved. The acidity or sourness of soils could not be measured by any one method which would indicate whether an injurious degree of acidity was in question, and it resulted that soil acidity became a broad biological concept, rather than a definite chemical one, and was judged in various ways; principally from consideration of (a) the effect of the soil on vegetation, (b) the effect of the soil on litmus paper, and (c) the drainage. It was commonly assumed that poor drainage was associated wtih an injurious degree of "acidity." It is true that a correlation has sometimes been found between poor drainage and high lime requirement; it is also true that in the experience of this laboratory the better drained soils have a higher hydrogen-ion concentration (a lower exponent) than the correlated, more poorly drained soils. To the mind of the writer it is very questionable whether the injurious effects of poor drainage on vegetation are in fact usually due to a high degree of acidity. It seems very probable that what is often called *sourness of soils* consists sometimes of a very *real* (i.e., injurious) acidity, but sometimes, namely when it is a case of poor drainage, of another property of soils, as yet not understood. Though at present it cannot be demonstrated, it seems more than likely that this other property of badly drained soils is their reducing power, and furthermore that the intensity aspect is likely to furnish a useful method of approach.

In short, it is suggested that the concept, *sourness of soils*, is ambiguous, referring to two different conditions; one, an acid condition, and another, not understood, but probably associated with high reducing potentials. Without doubt the word "acidity" is often used where the broader term "sourness" would be better justified.

THE SIGNIFICANCE OF REDUCING CONDITIONS IN SOILS

It appears to be well accepted that good soils are normally oxidizing, and that reducing conditions are an indication of an unhealthful state of affairs. The term "oxidizing" has been used in a broad sense by previous writers; and differences in degree of oxidation have been in terms of quantity, not of intensity. Reducing conditions have as a rule been recognized, either by the absence of oxidation, or by the presence of specific substances of known reducing power. Ferrous carbonate, which can be present only under reducing conditions, has been taken as the toxic agent concerned in badly drained soils. This can hardly be the only specific toxic reducing body present. A correlation has been noted between the occurrence of aldehydes and soil infertility (32), and a characteristic property of aldehydes is their reducing power. It has been found (18) that, in cane fields, soil from poor spots often showed greater reducing power for ferric salts than soil from good spots, and reference was made to the well-known reducing function of bacteria and of organic substances.

Honing (16) contrasted his unfavorable results, obtained with the method of Gerretsen (12), with the interesting results obtained by the latter. Honing states (in a summary in English):

> By this method Gerretsen could discern what soils were in good condition and what in a poor, not sufficiently oxydated one, as the soils with a high hydrogen-iodide value gave a rich crop of sugar or rice, and those with a low value, a small crop. . . . . When this method of testing irrigated soils on Java was tried on dry soils in Deli, it proved to be im-

> practicable . . . . the very high hydrogen-iodide value of soil samples taken 30–90 cm. beneath the surface is caused not by a *higher degree of oxydation* of the soil at that distance from the free air, but by the *large quantity* of oxydated material, i.e., ferric iron. This ferric iron is not equally divided over the whole depth. . . . .

This seems to be a clear illustration of the failure of a method based on the quantity factor alone.

In the light of the reduction-potential measurements of the writer, bacterial cultures are apparently normally reducing, and soils may become highly reducing if water-logged for a few days in the laboratory. It is very possible that similar changes will occur under field conditions, certainly under conditions of very poor drainage, and possibly, during periods of wet weather, in fields where the drainage is comparatively good. It would seem worth while to adopt the working hypothesis that a given intensity of reduction, as expressed by the reduction potential, has an equal biological effect in the soil, irrespective of the chemical reactions involved in producing the potential.

There can be no doubt that something is lacking in present methods of approach to the problem of soil aeration. Cannon and Free (4) state that "Although deficiency in aeration has frequently been suggested as an agricultural difficulty, or as the reason why certain species do not grow upon soils of heavy texture, it does not appear that this suggestion has had any exact experimental basis, ..." They appear to think that the question may be handled by means of gas analysis, but, just as the hydrogen-electrode potential is able to distinguish degrees of acidity and alkalinity in regions beyond the limits in which we can detect concentrations analytically, so is the reduction potential capable of distinguishing differences in degree of reduction in regions where oxygen can no longer be detected at all by gas analysis. If a curve is drawn, such as has been given by Wells (39), showing the concentrations of oxygen and hydrogen which would be in equilibrium with an unattackable electrode at various reduction potentials (in the presence of a suitable catalyst), it becomes obvious that the region capable of study by gasometric methods is only a small part of the possible field. Cannon and Free, however, made interesting observations by means of gasometric analysis; as did also Russell and Appleyard (30), who detected significant variations in certain patches of a field under different weather conditions. It may be doubted whether variations could be detected in this way in water-logged soils after any considerable period of immersion. In the cultivation of rice and other irrigated crops it is a matter of great importance to know when to remove the excess water. In a popular article (1) the importance of the subject has been recently emphasized, and the suggestion been made that practical application might be feasible of any principles which may be found.

We may interpret the fact that the quality of a soil is correlated with the quantity of substance produced in a test for oxidative power as follows. The bodies responsible for the reactivity of the soil in these tests may be supposed to be carriers of oxygen. The intensity factor of oxidation or reduction will not vary in great degree with changes in the concentration of these bodies, but they may protect the soil against reducing conditions for a limited time. When the soil is well aerated they are oxidized, and when the oxygen supply is too low they serve to supply oxygen to the soil constituents, so that a large supply of carriers is generally favorable.

Some soils become highly reducing when water-logged. The addition of organic substance aids in the production of reducing conditions, as reported above for peptone, and especially for dextrose. There can be no doubt that other organic substances have more or less action of this kind. Now, a considerable amount of work has been done upon the toxic effect of green manure in soils; this will not be reviewed here in detail. The subject has recently been discussed by Fred (10) in relation to the failure of seedlings. It is desired here to point out only that the toxic action of green manure is not well understood; it can in certain cases be attributed to infection of the plants with parasites (10), but naturally it is difficult to bring convincing proof as to whether the infection is the primary trouble, or a secondary one. It appears well worth consideration, whether the primary trouble may not be a high intensity of reduction.

SUMMARY

Oxidation and reduction potentials are discussed as the intensity factor of oxidation and reduction. Measurements of the reduction potentials of bacterial cultures or suspensions showed, when suitable precautions were taken, constant potentials for the facultative anaerobe *B. coli*, and for mixed cultures of soil microorganisms grown in a deep layer; the values for the reduction potentials being near the hydrogen electrode potentials. Measurements with aerobes (*B. subtilis*, *B. mycoides*, and a third aerobe), showed progressively increasing reduction potentials with lapse of time, but in no case did the reduction potential approach the hydrogen-electrode potential as closely as 0.3 volt. It is thought that this difference between anaerobes and aerobes may hold in general, but the evidence is not conclusive.

When soils were treated with an excess of water they became highly reducing, as evidenced by their reduction potentials. At the same time their hydrogen-electrode potentials changed. In the cases noted the changes of hydrogen-ion exponent indicated by the hydrogen-electrode potentials were very considerable, the soils becoming less intensely acid.[8] Accompanying the development of reducing conditions was a production in most cases of a foul odor.

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[8] Because of the abnormal properties of the water-logged soils, which might lead one to suspect a poisoning of the electrode (even though no evidence of poisoning was seen), no emphasis will be placed on this result at this time. The result seemed to the writer to require confirmation by the colorimetric method. However, Mr. L. A. Hurst of this laboratory has recently seen the same result, using the colorimetric method, in the case of a sample of Caribou loam.

Different soils differed in the rate at which they became highly reducing. The addition of dextrose to one soil favored the development of reducing conditions, the effect of 0.1 per cent of dextrose being very pronounced.

It is thought that "sourness" of soils includes something beyond acidity, and that the residual unfavorable quality may be a high intensity of reduction. A discussion is given of the significance of reducing conditions and of reduction potentials in soil study.

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FURTHER STUDIES ON THE FREEZING-POINT LOWERING OF SOILS AND PLANTS

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In an earlier contribution (1) we presented results of our investigations on the effect of certain environmental conditions on the freezing-point lowerings of the cell sap of the roots and tops of several crops. It was shown that the density of the cell contents of the leaves of plants increases from early morning until noon and again decreases in the evening, being lowest at night. The loss on drying at 100°C. was greatest at sunrise and in the evening. The increase in concentration of the cell sap, however, continues after the loss on drying becomes constant. On the other hand, when the plants are shaded the density tends to decrease during the day and no increase in the loss on drying is noted. As a result of greenhouse and field studies it was concluded that the concentration of the cell sap of the roots of plants is markedly and rapidly affected by changes induced in the concentration of the soil solution by the addition of soluble salts as well as a decrease in the water content of the soil mass. As the water content of the soil decreased the density of the cell sap of the roots increased, the freezing point lowerings approaching each other at the wilting point. On the other hand, the tops of plants were found to be less sensitive to the changes in the soil solution. Interesting data were obtained from crops grown on the same and different soils under field conditions. The dilatometer was employed in attempts to account for the daily fluctuations in the freezing-point lowerings of the cell sap of the leaves of plants. Less water was found to freeze in the barley tissue taken at noon than that sampled early in the morning.

It has been considered advisable to repeat some phases of these investigations and to continue others. Additional information has been obtained, some of which appears in this contribution.

WATER THAT FREEZES IN PLANT TISSUE UNDER DIFFERENT TEMPERATURES

The dilatometer has been employed in attempts to determine whether or not there is any relationship between the concentration of the soil solution, cell sap and the water that freezes in plant tissue at different temperatures. In making the determination, a sample of the fresh tissue is quickly weighed and inserted into the bowl of the dilatometer and ligroin added. Air bubbles are removed by stirring the tissue; the process may be hastened somewhat by means of a vacuum or suction pump. In determining the amount of water that freezes readily 10 gm. of the tissue may be used and the determination made in a freezing mixture of −1.5°C. The dilatometer is allowed to remain without agitation until the mass reaches the temperature of the bath, precautions being taken to prevent solidification. When this temperature is attained the dilatometer is agitated somewhat and solidification takes place. When equilibrium is established the reading is taken. It was found advisable to use a smaller amount of the tissue when employing markedly colder baths, inasmuch as there is a tendency for freezing to occur before the entire mass has reached the temperature of the bath. With some plants 2 gm. is the most practicable amount while with others four or five may be used.

Rye, wheat, corn, sweet clover and red clover were employed. The results presented in table 1 show the freezing-point lowerings of the leaves of several crops and also the amount of water that froze at temperatures of −1.5°C. and −4°C. In addition determinations were made on rye and clover leaves at temperatures of −2°C., −4°C. and −6°C., respectively. The results go to show that there are wide differences in the water that freezes in the different crops at the higher temperature; the crops that we used having the lowest concentration of cell sap as determined by the freezing-point method gave up the largest amounts The differences are less striking when the determinations are made at −4°C. or −6°C.

TABLE 1

The amount of water freezing in leaves of plants at different temperatures

CROP	DATE	WEIGHT OF MATERIAL	FREEZING-POINT DEPRESSION	− 1½°C	− 4°C	COOLED TO − 15°C WARMED TO − 4°C.	COOLED TO − 15°C. REFROZEN AT − 4°C.
		gm	°C	*cc*	*cc.*	*cc.*	*cc*
Rye . .	November 24	5	0 928	0 90	2 50	2 55	2 70
Rye. .	May 17	5	1 030	0 86	2 40		
Wheat	November 24	5	1 107	0 40	2 65	2 45	2 45
Sweet clover	November 24	5	0 906	1 22	2 82	2 61	2 61
Clover .	May 15	5	0 780	1 70	2 70		
Corn	June 10	5	0 578	2 10	2 90		

The amount of water that readily freezes in plant tissue should be of general interest to plant physiologists and it is probable that a knowledge of it would be valuable, especially where the changes in the concentration of the cell contents of plants as well as winter injury are being investigated.

The effect of low temperature also was determined. The dilatometer containing the tissue was placed in a bath the temperature of which was maintained at −15°C. until equilibrium was established, and then transferred to the −4°C. bath and the reading again taken when equilibrium was attained. Moreover, the tissue was frozen at −15°C., then thawed and refrozen at

−4°C. The low temperature increased somewhat the amount of water freezing in rye at −4°C., and decreased it somewhat in case of wheat and sweet clover. In preliminary experiments rye and clover leaves were subject to a temperature of −78°C. but the effect on the amount of water freezing at the higher temperature was slight. While more water undoubtedly froze at the lower temperature the concentration of the cell sap was such that thawing occurred when the material was warmed to −4°C.

EFFECT OF CONCENTRATION OF SOIL SOLUTION ON THE FREEZING-POINT LOWERINGS OF ROOTS AND TOPS OF PLANTS

The effect of the concentration of the soil solution on the freezing-point lowerings of the roots and leaves of plants at different periods was studied further. In addition the effect of this factor on the amount of water in the leaves freezing at −2.5°C. and −4.5°C. was investigated. The plants were grown in a house the roof and walls of which were made of oiled muslin. As shown in plate 2, the house was so constructed that the roof could be removed and the walls lowered, thus admitting direct sunlight and permitting a free circulation of air. With this arrangement it is believed that the conditions in so far as light and air are concerned are as nearly those of the field as can well be obtained.

A heavy sandy loam was used, the soil being packed into 3-gallon jars by hand so as to reduce variations to a minimum. Sufficient soil was added so that it could be rounded up above the sides of the jar. It was then covered with wax in order to prevent any rain which might by accident leak into the house, from entering the soil. Water was added through four ½-inch glass tubes which extended about an inch below the surface of the soil and 5 or 6 inches above, and also through an inverted funnel which reached about half way to the bottom of the jar. Under the bowl of the funnel and extending somewhat beyond it was placed a perforated paraffined cardboard plate to act as a spreader for the water. By this means a fairly uniform water content could be maintained in the soil.

The following concentrations of the soil solution were decided upon as giving a sufficient range:

FREEZING-POINT DEPRESSION	MATERIAL IN SOLUTION	OSMOTIC PRESSURE
°C	*p p m*	*atmospheres*
0.160	4000	1.930
0.317	7920	3.821
0.490	12250	5.904

In order to bring the soil solution to the concentration desired $MgSO_4$, KH_2PO_4 and $Ca(NO_3)_2$ were used in the proportion recommended by Shive. These salts were dissolved in distilled water and the resulting solution used to moisten the soil. The strength of the solution necessary to use to afford the desired concentration in the soil was determined by mixing a small portion of the soil with enough solution to give a moisture content of 15 per cent and determining the freezing-point lowering by means of the Beckmann thermometer. The concentration of the solution of the soil also was determined as it was placed in the jars.

The jars were taken down at 8 a.m. and samples of the leaves obtained for determining the loss upon drying, the freezing-point lowerings, and also for the measurement of the amounts of water freezing at different temperatures. The soil was then emptied from the jars and the roots hastily removed. A portion of the roots was freed as far as possible from soil by shaking and the sample placed in a freezing tube which was stoppered and packed in ice. Another portion of the roots was washed free of soil with distilled water and dried between filter paper, care being taken not to crush the roots. They were then placed in a stoppered tube and packed in ice until the determinations could be made. The soil was thoroughly mixed and samples drawn for moisture and freezing-point determinations.

TABLE 2

The concentration of the cell sap of the roots and tops of barley grown in soils of different concentration and the per cent of water in the leaves which freezes Period of growth 3 days

SOIL			TOPS				
Depression of freezing point	Osmotic pressure	Moisture content	Depression of freezing point	Osmotic pressure	Loss on drying at 100°C	Per cent of water freezing at	
						− 2 5°C	− 4 5°C.
°C.	*atmospheres*	*per cent*	°C	*atmospheres*	*per cent*		
0 100	1 209	15 53	0 909	10 948	87 74	52 92	70 56
0 284	3 423	13 88	0 949	11 428	88 12	55 10	73 87
0 499	6 012	14 47	0 995	11 980	87 63	54 01	69 61
0 112	1 350	15 70	0 847	10 210	88 05	57 32	73 87
0 255	3 074	15 33	0 832	10 527	88 38	57 32	73 86
0 379	4 567	15 81	0 905	10 90	87 95	55 10	72 77

UNWASHED ROOTS		WASHED ROOTS	
Depression of freezing point	Osmotic pressure	Depression of freezing point	Osmotic pressure
°C	*atmospheres*	°C	*atmospheres*
0 400	4 821	0 330	3 978
0 610	7 349	0 430	5 182
0 775	9 334	0 570	6 867
0 365	4 399	0 285	3 435
0 580	6 988	0 395	4 760
0 650	7 830	0 490	5 904

The data represent the average of duplicate determinations on the leaves of the plants and soil from each jar and where the amount of material on the roots permitted. The percentage of moisture in the tops and the amount of water freezing at different temperatures are calculated on the weight of fresh tissue.

The results obtained for barley after a 30-day period of growth are given in table 2. In the first set the plants in the jars with the lowest concentration of soil solution made the largest growth but were the worst infested with mildew. The plants grown in the soil of medium concentration were almost equal in height to the former while those grown in the soil of high concentration were much smaller.

In the second set the plants grown in the soil of medium concentration were more thrifty than those in the other jars. Where the soil had a low concentration the development was only slightly less, while in the jars of high concentration the plants were somewhat smaller and showed less root development.

The results in table 2 show a very slight tendency for the concentration of the cell sap of the tops of barley to increase as the concentration of the soil solution increases. The loss on drying at 100°C. and the percentage of water freezing as determined by means of the dilatometer likewise show little variation. More water froze at −4.5°C. than at −2.5°C , but there appears to be no relation between the amount of water freezing at either temperature and the concentration of the soil in which the plants were grown. On the other hand, the cell sap of the barley roots increased in concentration with each increase in concentration of the soil solution. This is true for both the washed and the unwashed roots

On account of the infestation with mildew it was not deemed advisable to continue the growth of barley for a longer period, and in consequence corn was substituted.

The results for corn after a 30-day period compose table 3.

There was not a great deal of difference in the growth of the corn in the soil with medium and with high concentration, but where the concentration was low the production was about 50 per cent less. This is well shown by plate 3, figure 1.

According to the freezing-point lowerings of the soil the concentration of the soil solution was materially lowered and consequently the variations in concentration were not so great as desired. Notwithstanding this lack of great variation in the osmotic pressure of the soil solution at the end of the period of growth, in the first series there was a decided increase in concentration of the cell sap of the roots with each increase in concentration of the soil solution. In the second series there was an increase in concentration of the sap of the roots of the plants grown in the second jar over those produced in the first but those in the third showed no greater lowerings than those of the second. It appears, therefore, that while the concentration of the soil solution at the time the experiment was completed did not vary as much as it did originally, the effects of the concentrations were still evidenced by the freezing-point lowering of the cell sap of the roots.

It is evident from the data obtained that the concentration of the cell sap of the tops of corn is not influenced so much by the concentration of the soil solution in which the plants are grown, as is the sap in the roots of the plants.

The variations in the moisture content of the tops were small. The per cent of water in the tops that froze at −2.5°C. did not change materially except in the case of the plants grown in the third jar, in which it was appreciably less. The amount of water that froze at −4.5°C. was greater than at −2.5°C., the plants grown in the third jar of each series giving up more than the others. Apparently there is no correlation between the concentration of the cell sap of the leaves and the per cent of water that freezes. This view is supported by the results reported in table 1.

TABLE 3

The concentration of cell sap of the roots and tops of corn grown in soils of different concentrations and the per cent of water in the tops which freezes. Period of growth 30 days

SOIL			TOPS				
Depression of freezing point	Osmotic pressure	Moisture content	Depression of freezing point	Osmotic pressure	Moisture content	Per cent of water freezing at	
						−2.5°C	−4.5°C
°C	*atmospheres*	*per cent*	°C	*atmospheres*	*per cent*		
0.085	1.0237	15.81	0.569	6.861	85.52	50.71	66.12
0.120	1.392	16.01	0.550	6.628	84.62	51.80	63.94
0.120	1.447	16.28	0.672	8.101	83.83	42.98	69.44
0.075	0.904	16.15	0.523	6.296	85.14	52.92	61.73
0.182	2.200	15.13	0.575	6.927	84.97	55.1	63.89
0.295	3.556	15.87	0.577	6.952	84.45	45.20	70.56

UNWASHED ROOTS		WASHED ROOTS	
Depression of freezing point	Osmotic pressure	Depression of freezing point	Osmotic pressure
°C	*atmospheres*	°C	*atmospheres*
0.245	2.953	0.250	3.014
0.387	4.670	0.313	3.779
0.577	6.957	0.390	4.700
0.422	5.091	0.275	3.315
0.590	7.108	0.380	4.580
0.577	6.957	0.375	4.519

As previously pointed out, the concentration of the soil solution in the various jars has been so reduced that the desired differences did not exist. It seemed advisable, therefore, to add sufficient quantities of the salt solution to maintain the original concentration. This was accomplished by frequently adding small quantities of the solution.

At the expiration of 60 days the corn in all cases had set ears. The silks were still fresh. The stalks in the jars of the lowest concentration were slender and the leaves of poor color. The plants growing in the soil of medium concentration were tall and sturdy and of good color. Where the high concentration was used, the stalks were larger in diameter than in the case of the jars of medium concentration, but much shorter. There was no apparent difference in the stage of maturity of the plants in the various jars. The variations in growth are shown by plate 3, figure 2.

TABLE 4

The concentration of the sap of the roots and tops of corn grown in soils of different concentrations and the per cent of water freezing Period of growth 60 days

SOIL			TOPS				
Depression of freezing point	Osmotic pressure	Moisture content	Depression of freezing point	Osmotic pressure	Moisture content	Per cent of water freezing at	
						− 2 5°C	− 4 5°C
°C	*atmospheres*	*per cent*	*°C*	*atmospheres*	*per cent*		
0 145	1 748	13 60	0 720	8 672	78 24	41 88	56 5
0 485	5 844	10 39	0 807	9 724	79 75	52 92	59 52
1 015	12 220	10 41	0 884	10 648	77 66	42 99	57 32
0 170	2 050	13 84	0 692	8 341	78 97	37 48	54 5
0 510	6 145	10 48	0 797	9 604	79 36	41 88	59 52
0 947	11 41	10 71	0 897	10 810	78 41	41 88	58 42

UNWASHED ROOTS		WASHED ROOTS	
Depression of freezing point	Osmotic pressure	Depression of freezing point	Osmotic pressure
°C	*atmospheres*	*°C*	*atmospheres*
0 560	6 747	0 225	2 712
1 080	13 00	0 562	6 777
1 157	13 93	0 842	10 147
0 540	6 507	0 362	4 3[illegible]9
1 115	13 42	0 595	7 168
1 062	12 7[illegible]	0 627	7 559

The results of the freezing-point, dilatometer and moisture determinations on the plants and soil for the 60-day period are given in table 4.

There was a decided increase in osmotic pressure of the cell sap of the tops with the increase in concentration of the soil solution, this being more apparent than in the previous series, or the one of shorter duration. The losses entailed on drying the tops at 100°C. show little variation Moreover, the water freezing at −2.5°C., except in the case of the plants grown in the second jar, did not vary a great deal. When a bath of −4.5°C. was employed a larger amount of water was frozen, as was noted in the 30-day series. The amount was somewhat less in the plants grown in the soils of the lowest concentration than in the others.

In case of the unwashed roots the freezing-point lowerings of the plants from the second and third jars were greater than those grown in the first.

In the second and third jars of each series the concentrations of the root sap of the unwashed roots appeared to be greater than that of the corresponding tops, an unusual condition.

The washed roots show a steady increase in concentration with each increase of osmotic pressure of the soil solution.

In order to make sure that the increase in concentration was not due to an accumulation of soluble material on the surface of the leaves rather than to material actually in solution in the cell sap, some of the leaves were washed with distilled water and sampled several hours later. The results obtained from the washed and unwashed leaves are set forth in table 5.

TABLE 5

Effect of washing on the concentration of the cell sap of corn leaves

SOIL		UNWASHED LEAVES		WASHED LEAVES	
Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic-pressure	Freezing-point depression	Osmotic pressure
°C.	*atmospheres*	°C	*atmospheres*	°C	*atmospheres*
0.145	1.748	0.720	8.672	0.645	7.770
0.485	5.844	0.807	9.724	0.710	8.552
1.015	12.220	0.884	10.648	0.712	8.576

These data show that when washed the correlation between the osmotic pressure of the cell sap of corn tops and that of the soil in which the plants were grown largely disappears. These results substantiate the conclusions of the writers in a previous paper (1), that the tops of plants are less sensitive to changes in concentration of the soil solution than are the roots.

THE EFFECT OF MOISTURE CONTENT OF SOILS ON THE CONCENTRATION OF THE CELL SAP IN ROOTS AND TOPS OF PLANTS

Under field conditions plants are subjected to extreme and quite rapid variations in the moisture content of the soil. To what extent the water content of the soils affects the osmotic pressure of the cell sap of the roots and tops of plants, as well as the loss on drying and the per cent of water freezing in the tops at different temperatures, is of interest. To obtain additional information on these points barley was grown in 3-gallon jars of sandy loam containing various amounts of moisture. The data obtained are presented in table 6.

No differences were found in the concentration of the cell sap or in the loss on drying of the tops of plants grown in soils of different moisture content. However, the roots, both washed and unwashed, possessed a higher concentration of cell sap when grown in the soil of lower water content. The per cent of water that froze in the tops was less in those plants grown in the soil with lower water content, both when a −2.5°C. and a −4.5°C. bath was used; that is to say, the water content of the soil mass in which the plant grew affected the form in which the water existed in the plant tissue.

TABLE 6

The concentration of the cell sap of roots and tops of barley grown in soils of different moisture content and the per cent of water in the leaves which freezes. Period of growth 30 days

SOIL			TOPS				
Depression of freezing point	Osmotic pressure	Moisture content	Depression of freezing point	Osmotic pressure	Moisture content	Per cent of water freezing at	
						− 2.5°C.	− 4.5°C.
°C.	*atmospheres*	*per cent*	°C	*atmospheres*	*per cent*		
0.012	0.141	23.29	0.903	10.880	88.36	57.87	81.21
0.100	1.205	15.53	0.090	10.948	87.79	52.92	70.56

UNWASHED ROOTS		WASHED ROOTS	
Depression of freezing point	Osmotic pressure	Depression of freezing point	Osmotic pressure
°C.	*atmospheres*	°C.	*atmospheres*
0.335	4.038	0.205	2.472
0.400	4.821	0.330	3.978

TABLE 7

Concentration of the cell sap of the roots and tops of corn grown in soils of different moisture content and the per cent of water in the leaves which freezes. Period of growth 60 days

Depression of freezing point	Osmotic pressure	Moisture content	Depression of freezing point	Osmotic pressure	Moisture content	Per cent of water freezing at	
						− 2.5°C	− 4.5°C.
°C.	*atmospheres*	*per cent*	°C.	*atmospheres*	*per cent*		
0.412	4.965	0.712	8.576	8.237	44.10	44.10	63.94
0.162	7.469	0.592	7.132	8.376	51.80	51.80	84.88

UNWASHED ROOTS		WASHED ROOTS	
Depression of freezing point	Osmotic pressure	Depression of freezing point	Osmotic pressure
°C.	*atmospheres*	°C.	*atmospheres*
0.614	7.406	0.419	5.055
0.357	4.301	0.279	3.369

The results obtained for corn after 60 days' growth are to be found in table 7.

These data substantiate in general the conclusions drawn from the results obtained from the previous series. The increase in the amount of water that froze in the tops at −2.5°C. with an increase in water content of the soil was not so regular as in the earlier series. The amount of water freezing at −4°C., however, varied with the moisture content of the soil. In considering these data it must be remembered that while the concentration of the soil solution in the soil of each jar was quite low, yet that in the soil containing approximately 15 per cent of moisture was about ten times as dense as that of the soil containing 23 per cent. It must be admitted, therefore, that it is not clear from this series whether the differences in concentration of the cell sap of the roots is due to the differences in water content of the soil or the variation in concentration of the soil solution.

In order to determine the effect of different water contents of the soil on the concentration of the roots and tops of the plants growing in it, the concentration of the soil solution remaining the same, the next series of experiments was conducted. Jars of sandy loam were made up to 10, 15 and 22½ per cent moisture content and sufficient salts (Shive's 3-salt nutrient solution) added to the soil containing 15 and 22½ per cent water to give it practically the same concentration as that containing 10 per cent moisture.

The concentrations in the various jars at planting were:

	Freezing-point depression °C.
10 per cent H_2O jars	0.238
15 per cent H_2O jars	0.250
22½ per cent H_2O jars	0.260

The results for corn after a period of 30 days' growth are shown in table 8.

Notwithstanding the addition of salts the soil with high moisture content had less depression at the conclusion of the experiment than the soil of low water content. On the other hand, the concentration of the soil solutions in the soils of medium and of low water content approached sufficiently near to each other to allow of some conclusions being drawn from the results.

There is very little relation between the moisture content of the soil and the concentration of the cell sap of the tops and the moisture content of the tops of the plants grown in soil of low and of medium water contents. The per cent of water in the tops that froze at −2.5°C. increased as the water content of the soil decreased. When a bath of −4.5°C. was used, however, there was very little difference in the percentage of water that froze. These results are unlike those obtained from previous series in which the water content of the soil remained constant and the concentration of the soil solution varied, or the one in which the moisture content of the soil varied, no effort being made to control the concentration of the soil solution. This is quite interesting, as it raises the question of the influence of soluble salts in the soil solution on the free water in plant tissue with soils of varying moisture contents.

TABLE 8

Concentration of the cell sap of roots and tops of corn grown in soils of different moisture content the concentration of soil solution remaining constant. Period of growth 30 days

SOIL			TOPS				
Depression of freezing point	Osmotic pressure	Moisture content	Depression of freezing point	Osmotic pressure	Moisture content	Per cent of water freezing at	
						−2.5°C.	−4.5°C.
°C.	*atmospheres*	*per cent*	°C.	*atmospheres*	*per cent*		
0.097	1.145	23.50	0.625	7.529	85.66	41.88	59.52
0.310	3.736	15.31	0.642	7.740	86.58	46.30	63.94
0.242	2.923	10.77	0.622	7.499	84.87	52.92	63.94
0.137	1.603	21.00	0.632	7.619	85.75	41.88	63.94
0.232	2.802	15.34	0.650	7.830	86.01	44.10	61.74
0.275	3.315	9.86	0.605	7.289	85.18	52.92	61.18

UNWASHED ROOTS		WASHED ROOTS	
Depression of freezing point	Osmotic pressure	Depression of freezing point	Osmotic pressure
°C.	*atmospheres*	°C.	*atmospheres*
0.410	4.941	0.320	
0.525	6.326	0.415	5.001
0.672	8.101	0.370	4.459
0.440	5.302	0.365	4.399
0.525	6.326	0.425	5.122
0.665	8.010	0.460	5.543

SUMMARY

The amount of water that froze readily, or at −1.5°C. and at lower temperatures in the leaves of several crops, was determined. Wide variations in the amount of water that easily froze in the plants studied were found, the greatest taking place in those having low freezing-point depressions. The differences were much less striking when lower temperatures were employed. Larger amounts of water were found to freeze when lower temperatures were employed. However, subjection to very low temperature (−15°C.) resulted in the freezing of no more water at −4°C. when subsequent determinations were made at that temperature.

Considering all the data presented, the conclusion seems justified that the concentration of the cell sap of the roots of the plants used in these investigations is influenced quite decidedly by the concentration of the soil solution in which the plants are grown. While it is probable that determinations made on either the washed or unwashed roots do not represent the true concentration of the cell sap and while there are some variations in the results obtained, yet considering the data as a whole, the above conclusion is warranted. The effect of the concentration of the soil solution was not so marked on the concentration of the cell sap of the leaves.

These results substantiate the conclusions drawn from work previously published.

Marked increases in the concentration of the soil solution induced by the addition of full nutrient solution of varying strength, the water content of the soil remaining constant, did not measurably alter the amount of water that froze at −2.5°C. and −4°C., respectively. If higher temperatures had been employed the results might have been somewhat different.

Corn and barley plants grown in a soil of high, of medium and of low water content possessed more easily freezable water when grown in the soil of higher water content. When the water content varied and the concentration of the soil solution was maintained practically constant by the addition of a nutrient solution, more water froze at −2.5°C. in the leaves of the plants grown in the soils of low water content. There was very little difference in the amount of water that froze at −4°C. These results are quite interesting and somewhat at variance with the results obtained when both the water content of the soil and the concentration of the soil solution varied.

It is desired to call attention to the possibility of utilizing the dilatometer method for investigations in plant physiology.

REFERENCE

(1) McCool, M M , and Millar, C. E. 1917 The water content of the soil and the composition and concentration of the soil solution as indicated by the freezing-point lowerings of the roots and tops of plant. *In* Soil Sci., v. 3, no. 2, p. 113.

PLATE 1

Fig 1. Plant house closed. Front view.
Fig. 2. Plant house closed. Rear view.

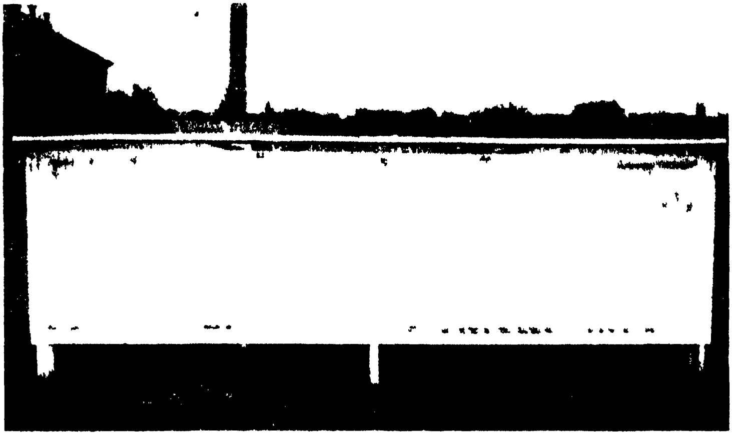

FIG. 1

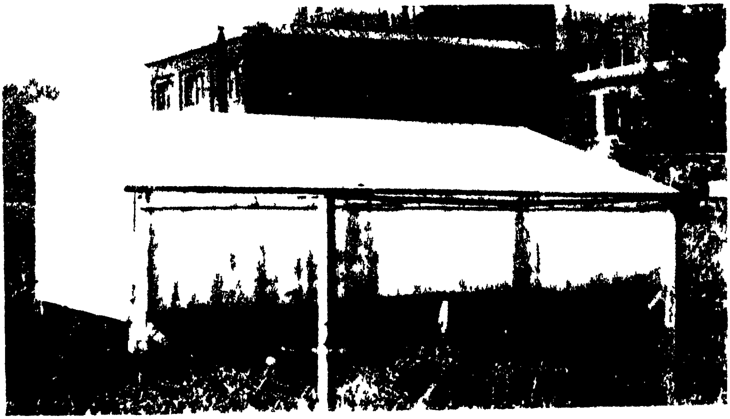

FIG. 2

PLATE 2

Plant House Open

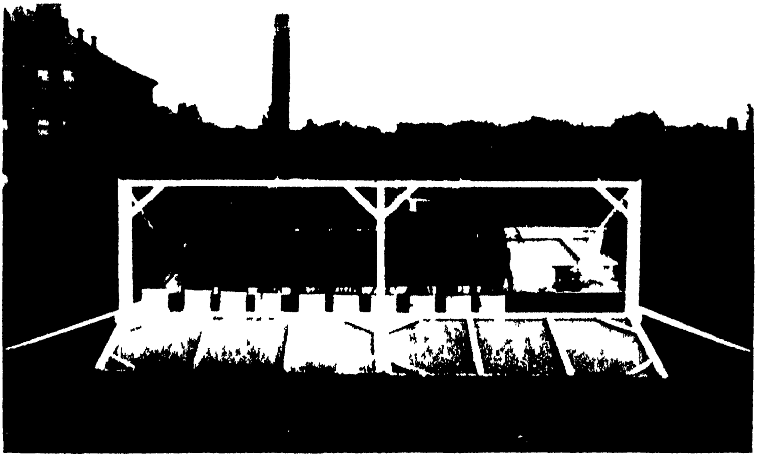

PLATE 3

Fig. 1. Corn. Period of growth 30 days. Osmotic pressure of soil solution at time of planting: No. 1, 1.193 atmospheres; No. 2, 3.821 atmospheres; No. 3, 5.904 atmospheres.

Fig. 2. Corn. Period of growth 60 days. Osmotic pressure of soil solution: No. 1, 1.748 atmospheres; No. 2, 5.841 atmospheres; No. 3, 12.220 atmospheres.

FREEZING POINT LOWERING OF SOILS AND PLANTS PLATE

M M M L ANG F MILLAR

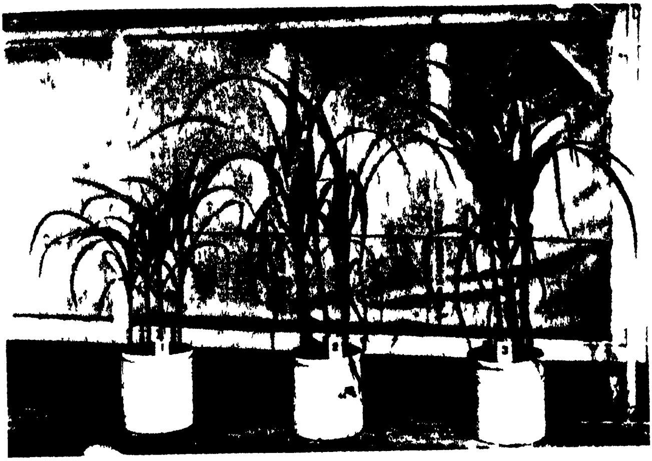

Fig. 1

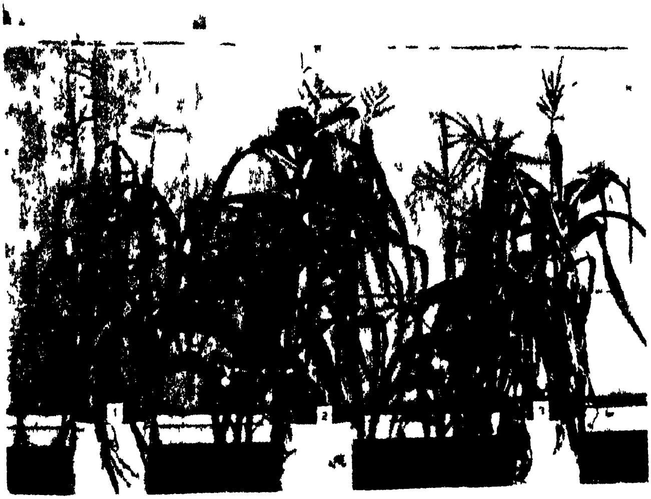

Fig. 2

THE EFFECT OF LEACHING ON THE AVAILABILITY OF ROCK PHOSPHATE TO CORN[1]

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Insoluble plant-food materials become available to the plant only through the influence of some active agent The carbonic acid in the soil produced by the growing plant and by biological processes, is no doubt the most important agent. The action of carbonic acid on insoluble plant-food materials, such as rock phosphate, follows the laws of mass action and chemical equilibrium, and may be represented as follows:

$$Ca_3(PO_4)_2 + 2H_2CO_3 \rightleftarrows Ca_2H_2\,(PO_4)_2 + CaH_2(CO_3)_2$$

Any changes of the masses or concentrations of the compounds of this system will cause a change in the equilibrium. If a plant removes both of the soluble products formed, in the proportion produced, the reaction will proceed indefinitely. If, however, the plant removes the soluble phosphate and only a lesser proportion of the calcium bicarbonate, a point of equilibrium will be established in which there will be proportionately more of the soluble calcium bicarbonate present. The continued removal of the soluble phosphate and only a partial removal of the calcium bicarbonate will finally result in such a large accumulation of the calcium bicarbonate and a consequent change in the equilibrium that the concentration of soluble phosphate will become insufficient for plant growth. On the basis of these considerations, Truog (9) has developed his theory in regard to the feeding power of plants for insoluble phosphates, which is stated as follows:

> Plants containing a relatively high calcium oxide content have a relatively high feeding power for the phosphorus of rock phosphate For plants containing a relatively low calcium oxide content, the converse of the above is true. The explanation of this relation is made possible by means of the laws of mass action and chemical equilibrium.

Chirikov (3) also believes that there is a direct correlation between the calcium needs of the plant and its ability to use insoluble phosphates.

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[1] Part of a thesis submitted at the University of Wisconsin in partial fulfillment of the requirements for the Degree of Doctor of Philosophy. Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

The writer wishes to express his appreciation for the helpful suggestions and criticisms tendered by Prof. E. Truog.

In view of this theory, it appears that if in some manner the soluble calcium bicarbonate could be entirely or in part removed by leaching, or even moved about in the soil medium or made more soluble through the use of certain salts, equilibrium would at least not be established so quickly and it would be possible for a relatively weak feeding plant to utilize insoluble phosphates to a greater degree. An acid condition of the soil which gives a certain capacity to combine with basic materials and hence remove calcium bicarbonate from solution, has been shown by Prianichnikov (6) Kossowitch (4) and others to increase the availability of insoluble calcium phosphates to many plants.

EXPERIMENTAL

Plan of work

The investigations reported herein were planned primarily to test the effect of leaching on the availability of rock phosphate. As a test crop, corn was selected because it nicely fulfills the requirements of being a poor feeder on insoluble phosphates, uses only a little calcium, and is a comparatively rapid grower.

The plants were grown in a greenhouse in 2-gallon, glazed earthenware pots. Each pot was provided with a ⅜-inch hole on the side just above the bottom for drainage. In the unleached pots these holes were partly closed with a cork stopper in which a small groove had been cut to facilitate air movement, and to carry away any excess water that might be present. To facilitate drainage in the leached pots these holes were provided with rubber stoppers, through which passed a slightly bent glass tube, protected on the inside by a small bunch of glass wool. To each pot was added 11,000 gm. of pure white quartz sand with which the phosphate treatments indicated in table 1 had been thoroughly mixed.

Five seeds of a standard variety of corn were planted in each pot and later thinned to the best three plants. All treatments were duplicated.

The nutrient solution used was made up from single-salt stock solutions which were prepared by dissolving separately in liter portions of water, 68 gm. of sodium nitrate, 32 gm. of ammonium nitrate, 20 gm. of potassium sulfate, 8 gm. of magnesium sulfate, and 0.4 gm. of ferric chloride. The standard application of these solutions was 10 cc. of the first four, and 1 cc. of the last, diluted as desired, usually to 1 liter. The two solutions of nitrogen salts indicated, carried the same amount of nitrogen. Only one form of nitrogen salt was used on a single culture, as indicated in table 1. After growth was well started, applications were made once each week. Both the phosphorus and calcium had to be secured by the plant from the calcium phosphates mixed in the sand. Calcium nitrate was applied to the check pot.

The moisture content of the unleached pots was maintained by frequent weighings, at about 13 per cent of the weight of the sand. No attempt was made to maintain a definite moisture content in the leached pots, as the plants were grown under well drained conditions with sufficient moisture.

Each week the one set of pots was leached with the nutrient solution until approximately 1 liter of drainage water was secured from each pot. Later these leachings were made twice a week. The solutions from the leachings were filtered immediately through Pasteur-Chamberland clay filters, and the filtrates retained for determinations of the calcium content.

The plants were harvested just as the tassels were beginning to appear. One set of duplicates was used after securing the green weights for determinations of the plant sap acidity which is to be reported in another paper (1). A sample from each pot was retained for dry-matter determinations. The other set was harvested, and all the material was weighed and dried for dry-matter content and chemical analysis. The general appearance at harvest time is shown in plate 1.

Effect of treatments on plant growth

The data of weights indicating growth are summarized in table 1, and are graphically shown in figure 1.

Leaching the soil had a decided influence upon the availability of the rock phosphate to the corn plant. This was true where either form of nitrogen salt accompanied the rock phosphate. The smallest yield was secured from the unleached pot (no. 39) treated with $NaNO_3$. With this treatment the soil solution probably became saturated with calcium bicarbonate, which greatly hindered the further solution of the phosphate, as explained by the laws of mass action and chemical equilibrium. Evidently the nutrient solution containing the $NaNO_3$ had little or no solvent effect on the rock phosphate. The data for the leached pot (no. 47) show a striking increase in yield. Under these conditions the soil solution was freed from the excessive amounts of calcium bicarbonate, and in accordance with the laws of mass action and chemical equilibrium, more phosphate was brought into solution. This increase in growth is more clearly brought out in plate 2 which shows these plants after the sand had been washed out of the roots.

Analogous results were secured when NH_4NO_3 was used with rock phosphate instead of $NaNO_3$. The yields, however, were much higher for both the leached and the unleached pots. The increase due to leaching under these conditions was very much greater than when $NaNO_3$ was used. These differences could have been due only to the influence of NH_4NO_3. Evidently this salt increased the solubility of the calcium bicarbonate more than $NaNO_3$; it thus changed the point of equilibrium and caused the reaction making the rock phosphate available to proceed with greater rapidity. The NH_4NO_3, as is indicated further on, probably also acted as a physiologically acid salt. The use of NH_4NO_3 thus allowed greater growth in both the leached and the unleached pots than $NaNO_3$. The greatest growth of all was produced with a combination of rock phosphate, NH_4NO_3 and leaching (pot 51).

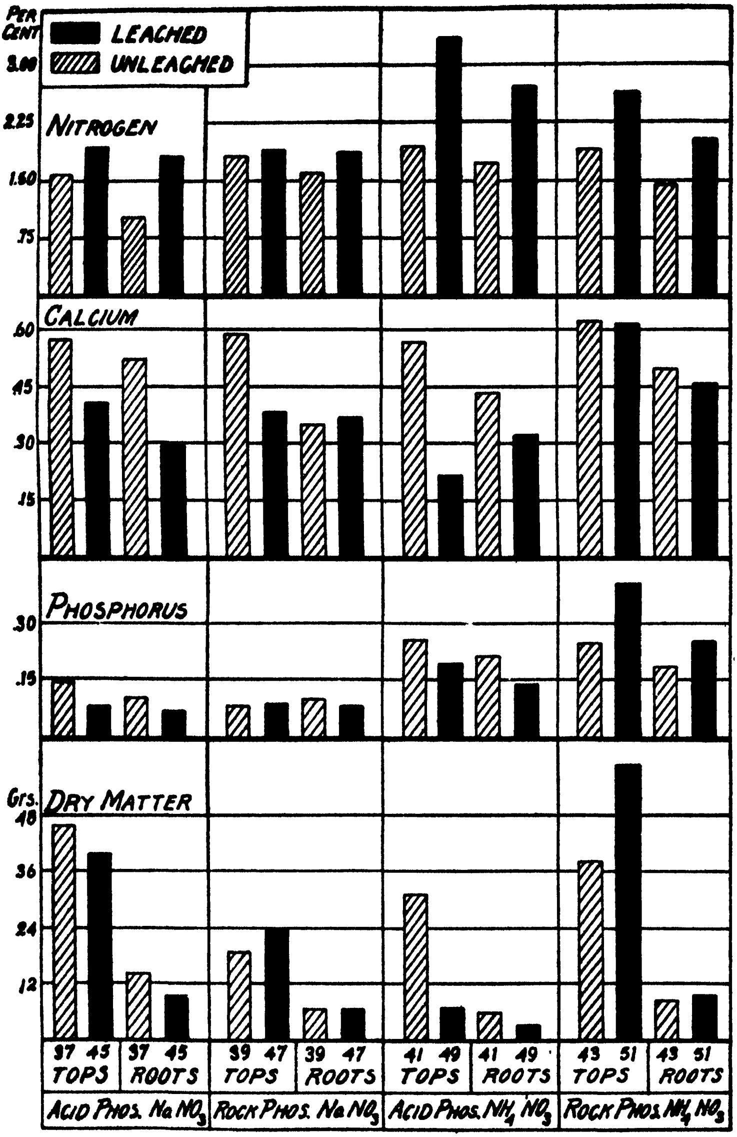

FIG. 1. EFFECT OF LEACHING ON THE WEIGHT OF DRY MATTER, AND CONTENT OF PHOSPHORUS, CALCIUM AND NITROGEN OF CORN GROWN WITH THE TREATMENTS INDICATED

Leaching the pots treated with acid phosphate gave poorer results than no leaching. In fact, the results due to leaching with acid phosphate are just the opposite to those secured with rock phosphate. With $NaNO_3$ leaching definitely decreased the yield but not excessively. With NH_4NO_3, however, leaching very decidedly decreased the yield, and the poorest yield of all cultures, outside the check pot, was produced in the leached pot (no. 49) where acid phosphate was used with NH_4NO_3. Here it appears, as will be shown in the paper on the hydrogen-ion concentration of the plant juices and the soil solution (1), that leaching increased the acidity sufficiently to make conditions unfavorable for plant growth.

TABLE 1

The green and dry weights, water content, and comparative dry weights of corn produced with treatments indicated

POT NO	TREATMENT*	AVERAGE WEIGHTS OF DUPLICATES				COMPARATIVE DRY WEIGHT† OF TOPS	WATER AS PER CENT OF GREEN WEIGHT	
		Green		Dry				
		Tops	Roots	Tops	Roots		Tops	Roots
		gm	*gm*	*gm*	*gm*		*per cent*	*per cent*
35	Check	7 0	18 1	1 9	1 2	4 2	73 6	93 1
37	Acid phosphate, $NaNO_3$, unleached .	236 9	146 2	45 2	13 3	100 0	80 9	90 0
45	Acid phosphate, $NaNO_3$, leached	225 2	113 0	39 0	8 4	86 3	82 7	92 6
39	Rock phosphate, $NaNO_3$, unleached .	72 0	82 6	18 3	6 2	40 5	74 6	92 4
47	Rock phosphate, $NaNO_3$, leached	126 9	99 9	23 4	6 9	51 7	81 5	93.1
41	Acid phosphate, NH_4NO_3, unleached	209 6	92 6	31 2	6 0	69 0	85 1	93 6
49	Acid phosphate, NH_4NO_3, leached .	68 8	53 8	7 0	3 0	15 4	89 7	94 5
43	Rock phosphate, NH_4NO_3, unleached	194 4	74 0	38 8	8 2	85 8	80 0	88 9
51	Rock phosphate, NH_4NO_3, leached	308 3	133 3	58 9	10 5	130 3	80 9	92 1

* Phosphate applications on the acre basis were 400 pounds of acid phosphate and 2000 pounds of rock phosphate.

† Growth with treatment of acid phosphate, $NaNO_3$ and no leaching, is represented by 100.

The effect of leaching where acid phosphate was used was evidently not a question of removing soluble calcium bicarbonate, as is the case where rock phosphate was used, but was one of changing the environment in other ways, which here appeared to have rather marked injurious effects. These injurious conditions probably resulted from a lack of sufficient basic material.

Effect of treatments on plant composition

Leaching the soil also increased the water content of the plants. This was probably due to the decrease in the concentration of salts in the soil which favored greater availability of water to the roots. The percentage of water in the roots was greater than in the tops.

Table 2 gives the content of phosphorus, calcium and nitrogen as determined by analysis of the tops and roots of the plants grown with the different treatments. These results along with the dry weights are shown graphically in figure 1, where comparisons of the effect of the various treatments may easily be made.

TABLE 2

The content of phosphorus, calcium, and nitrogen in corn plants grown with the treatments indicated. Data are averages of duplicates

POT NO	TREATMENT*	PHOSPHORUS		CALCIUM		NITROGEN	
		Tops	Roots	Tops	Roots	Tops	Roots
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
35	Check...	0 074	0 063	0 835		2 11	0.60
37	Acid phosphate, $NaNO_3$, unleached . .	0 135	0 102	0 571	0 522	1 59	1 02
45	Acid phosphate, $NaNO_3$, leached.	0 086	0 075	0 407	0 297	1 95	1 84
39	Rock phosphate, $NaNO_3$, unleached	0 083	0 101	0 558	0 355	1 85	1 61
47	Rock phosphate, $NaNO_3$, leached	0 088	0 084	0 386	0 370	1 92	1 85
41	Acid phosphate, NH_4NO_3, unleached .	0 258	0 212	0 553	0 433	1 96	1 73
49	Acid phosphate, NH_4NO_3, leached .	0 195	0 132	0 219	0 313	3 38	2 74
43	Rock phosphate, NH_4NO_3, unleached.	0 248	0 187	0 616	0 501	1 90	1 43
51	Rock phosphate, NH_4NO_3, leached	0 402	0 248	0 611	0 459	2 66	2 07

* Phosphate applications on the acre basis were 400 pounds of acid phosphate and 2000 pounds of rock phosphate

Just as there was a wide variation in the amounts of dry matter produced, there was also a wide variation in the contents of phosphorus, calcium and nitrogen. The degree and direction of the variation, however, were not the same for the three elements.

Leaching the soil increased the phosphorus content of the corn plant when rock phosphate was used, and decreased it when acid phosphate was used. The highest content of phosphorus occurred with NH_4NO_3. With $NaNO_3$ the phosphorus content was rather uniform. In general, the percentage content of phosphorus varied in the same direction as growth, but with smaller fluctuations.

The calcium content of the corn plants was in most cases greatly decreased by leaching. The increased growth due to leaching, when rock phosphate was used, was accompanied by a higher phosphorus content, and in general, a lower calcium content. This is to be expected when the availability of rock phosphate is considered in connection with the laws of mass action and chemical equilibrium.

The calcium content of the tops from all unleached pots was quite uniform. When $NaNO_3$ was used the calcium content of the tops from leached pots shows but slight variation, but when NH_4NO_3 was used the results are quite divergent. The use of acid phosphate and NH_4NO_3 with leaching (pot 49) gave the lowest calcium content of tops of all cultures, and the use of rock phosphate, under similar conditions (pot 51), gave the highest calcium content.

The nitrogen content of the corn plant was increased by leaching. This increase was expecially marked when NH_4NO_3 was used. With no leaching the nitrogen content of all plants was quite uniform. Just why leaching should have caused the plant to take up more nitrogen, with no definite relation to growth, is not clear. With the NH_4NO_3 it may be that the NH_4 ion partly functioned as a base in place of the calcium. With acid phosphate the calcium content was very low, and this seems much more probable than with rock phosphate, in which case the plant appears to have had all the calcium it needed. This explanation is in harmony with the calcium content of the corn plant as noted above.

Effect of treatments on the calcium content of the drainage waters

Table 3 presents the data showing the soluble calcium derived from the rock phosphate and removed by leaching. Figure 2 graphically shows the influence of NH_4NO_3 as compared to $NaNO_3$ on the formation of soluble calcium, and hence availability of rock phosphate. It will be noted that the first leaching in both cases gave a rather large amount of soluble calcium. This, no doubt, represents the calcium of the rock phosphate that is readily soluble, and explains why there is but little difference with the two salts in the first leaching. After the first leaching the influence of the NH_4NO_3 on the solubility of the calcium becomes more and more marked.

The influence of $NaNO_3$ and NH_4NO_3 on the availability of rock phosphate

The results in table 3 are in harmony with the theory of the availability of rock phosphate in relation to the laws of mass action and chemical equilibrium. Sodium nitrate has little or no influence on the reaction, while NH_4NO_3 gives rise to conditions which increase the solubility of the calcium and change the point of equilibrium to a marked extent. This increases the solubility of the phosphorus in the rock phosphate and allows greater plant growth. When these larger amounts of calcium bicarbonate and other soluble calcium salts are removed by leaching, the availability of the rock phosphate and consequently plant growth, is further greatly accelerated. It will be noted that

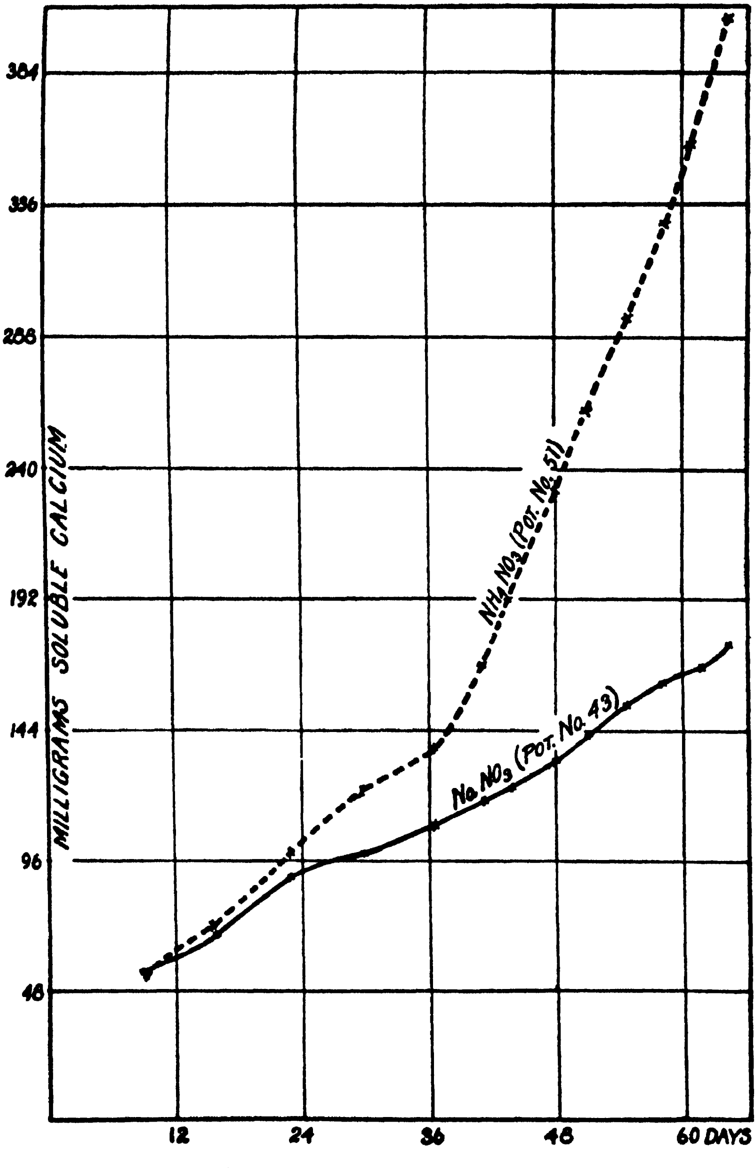

FIG. 2. INFLUENCE OF NH_4NO_3 COMPARED WITH $NaNO_3$ ON THE LEACHING OF CALCIUM BICARBONATE FROM ROCK PHOSPHATE IN CORN CULTURES

practically three times (actually 2.9) as much calcium was leached from the pot (no. 51) containing NH_4NO_3 as out of the similar pot (no. 47) containing $NaNO_3$. The growth in the former was 2.5 times as great, which is in rather close agreement with the removal of calcium.

The influence of NH_4NO_3 on the availability of rock phosphate has been observed by Kossowitch (5), Prianichnikov (7) and Shulov (8), who explain it as due entirely to the action of NH_4NO_3 as a physiologically acid salt. Burlison (2), on the other hand, who used NH_4NO_3 in his nutrient solution, states that the solution had no appreciable influence on the availability of the rock phosphate in his experiments.

TABLE 3

The amounts of soluble calcium derived from rock phosphate and removed by leaching

NUMBER OF DAYS FROM BEGINNING OF EXPERIMENT	TOTAL CALCIUM REMOVED FROM EACH POT		RATIO OF SOLUBLE CALCIUM REMOVED BY $NaNO_3$ AND NH_4NO_3
	$NaNO_3$	NH_4NO_3	
	mgm.	*mgm*	
9	53.88	53.37	1.0
16	13.63	18.75	1.3
23	21.63	27.02	1.2
30	10.38	23.69	2.3
37	8.25	15.14	1.8
41	9.37	30.10	3.2
44	7.45	27.32	3.6
48	7.11	36.19	5.0
51	10.81	31.72	3.0
55	11.09	33.02	3.0
58	5.70	31.21	5.4
62	8.09	30.50	3.8
65	9.09	46.18	5.0
Total............	176.48	404.21	2.3
Total without first....	122.60	350.84	2.9

There is no question, however, from the results reported herein, but that the NH_4NO_3 did have a marked influence on the availability of the rock phosphate.

As has been briefly indicated, the favorable influence of the NH_4NO_3 on the availability of the rock phosphate may have been due to its effect on the solubility of the calcium bicarbonate, or to the increased acidity resulting from nitrification or more rapid removal of the NH_4-ion than of the NO_3-ion by the growing plant, or to all these factors combined. The sharp upward bend in the curve for the NH_4NO_3 after about 36 days of plant growth, shown in figure 2, indicates that the production of soluble calcium was not alone dependent upon the direct chemical action of NH_4NO_3. Evidently the biological factors acting on NH_4NO_3 became more important at this point. This is substantiated by the determinations of the hydrogen-ion concentrations or acidity which will be reported in a later paper (1). In all cases the soil medium and plant juices were more acid when NH_4NO_3 was used.

In order to determine more fully the comparative direct chemical effect of the nutrient solutions containing either NH_4NO_3 or $NaNO_3$ on the solubility of calcium bicarbonate and availability of rock phosphate, a battery of six percolators was arranged, in which distilled water and the complete nutrient solutions, containing either $NaNO_3$ or NH_4NO_3, were allowed to act with and without carbon dioxide on rock phosphate for 48 hours at room temperature. Each percolator contained 600 cc. of either distilled water or nutrient solution and 4.6 gm. of rock phosphate, which is the same proportion in which these materials were used in the pot cultures. Effective agitation was maintained in the one case by the bubbling of CO_2 from a tank, and in the other by the aspiration of CO_2-free air. The results obtained are given in table 4. A direct comparison of the solubility effects on rock phosphate of either $NaNO_3$ or NH_4NO_3 in conjunction with other nutrient salts may be made by subtracting the amounts of soluble phosphorus and calcium produced with the distilled or carbonated water from the amounts of these soluble materials produced when the nutrient salts were added to these. Figures from such subtractions are given in table 5.

TABLE 4

Soluble phosphorus and calcium in successive extractions of rock phosphate with the solutions indicated

TREATMENTS	FIRST EXTRACTION		SECOND EXTRACTION		THIRD EXTRACTION		TOTAL FOR THREE EXTRACTIONS	
	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus	Calcium.
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
H_2O . . .	1.92	9.62	0.8	6.41	1.28	8.02	4.00	24.05
Nutrient solution with $NaNO_3$.	2.16	17.64	0.56	8.82	1.76	13.13	4.48	39.59
Nutrient solution with NH_4NO_3. .	1.56	19.25	1.00	10.43	1.68	11.63	4.24	41.31
H_2O with CO_2	5.84	28.07	5.00	17.64	4.80	19.25	15.64	64.96
Nutrient solution with $NaNO_3$ and CO_2	7.04	34.49	5.28	22.45	5.88	22.86	18.20	79.80
Nutrient solution with NH_4NO_3 and CO_2.	7.76	42.50	5.48	23.26	5.70	24.46	18.94	90.22

TABLE 5

The comparative solubility effects on rock phosphate of nutrient salts containing nitrogen as $NaNO_3$ *and* NH_4NO_3 *calculated from data in table 4*

NUTRIENT SALTS	WITHOUT CO_2		WITH CO_2	
	Phosphorus	Calcium	Phosphorus	Calcium
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
Salts containing $NaNO_3$. . . .	0.48	5.54	2.56	14.84
Salts containing NH_4NO_3	0.24	7.26	3.30	25.26

The nutrient solution containing either $NaNO_3$ or NH_4NO_3 increased the availability of the rock phosphate to some extent over that of water. Without CO_2 there was but little difference in the effect of the two salts. With the addition of CO_2, which makes the conditions more closely comparable to those in pot cultures, the NH_4NO_3 was more effective than the $NaNO_3$, especially in the production of soluble calcium. From the data presented it appears that the influence of NH_4NO_3 upon the availability of rock phosphate is only in part due to its effect on the solubility of the calcium bicarbonate. Its capacity to produce an acid medium, as has been found, by acting either as a physiologically acid salt or by becoming nitrified, is probably an important factor in this connection.

GENERAL DISCUSSION

The data presented herein show that leaching the soil increases the availability of rock phosphate to corn. This fact is manifested by increased growth and a higher percentage content of phosphorus in the plant. Accompanying the increase in growth, there is found a proportionate increase of calcium in the solutions from the leachings and a lower percentage content in the plant. These results are readily explained by the laws of mass action and chemical equilibrium, and are in harmony with Truog's theory (9) concerning the utilization of insoluble phosphates by crops.

Carbonic acid is no doubt the principal agent in these reactions. The soluble calcium in the leachings and the results obtained indicate this. The main source of carbonic acid in quartz cultures of this kind is that which is excreted by the growing plant through its roots. As it is formed and comes in contact with the rock phosphate both soluble phosphorus and soluble calcium bicarbonate are produced. These compounds are in equilibrium with the insoluble phosphate and the carbonic acid. The corn plant has greater need proportionally for the soluble phosphate than it has for the soluble calcium; hence the soluble phosphorus is assimilated and much of the calcium is left behind. As the calcium bicarbonate accumulates in the soil it tends to bring about equilibrium and thus suppress the formation of soluble phosphorus. When this condition exists, the plant has access to large amounts of available calcium but little phosphorus, and consequently the plant shows a high calcium and low phosphorus content and a stunted growth. When the soil is leached the excess of soluble calcium bicarbonate is removed from the root zone and thus the point of equilibrium in the reaction with rock phosphate is not so rapidly attained, and more phosphorus becomes available to the plant. Greater growth results and the plant shows a higher phosphorus and a lower calcium content. Plants that have a relatively large need for calcium would therefore be better feeders on rock phosphate than those that have but little need for calcium, as pointed out by Truog.

The data show further that accompanying fertilizers may directly increase the solubility of the calcium bicarbonate or even produce an acid medium and thus increase the availability of the rock phosphate by delaying the rapidity with which the equilibrium is established. Ammonium nitrate as compared with sodium nitrate shows marked influences in these respects. Nearly three times as much soluble calcium was found in the leachings from the NH_4NO_3-treated pots as in those from the $NaNO_3$-treated pots. This indicates that there should be a proportionate increase in the soluble phosphorus formed and consequently greater growth. The results show that growth increased in about the same ratio.

SUMMARY

1. Leaching the soil increased the availability of rock phosphate to corn by removing the excess of soluble calcium bicarbonate and other soluble calcium salts. These results are in accord with the laws of mass action and chemical equilibrium.
2. Ammonium nitrate had a marked influence on the solubility of rock phosphate to corn, due to its favorable effect on the solubility of calcium bicarbonate, and its capacity to produce an acid medium either by acting as a physiologically acid salt or by becoming nitrified.
3. Sodium nitrate had no appreciable influence on the availability of rock phosphate.
4. The soluble calcium leached from the pots treated with rock phosphate under the influence of $NaNO_3$ and NH_4NO_3 was found to be in the ratio of 1:2.9. The growth ratio of corn under the same conditions was 1:.2.5, showing therefore, a rather definite relation between the calcium leached out in solution and plant growth.
5. Leaching caused a decrease in the production of dry matter where acid phosphate was used.
6. Ammonium nitrate with acid phosphate appeared to cause a physiological disturbance in the plants, especially with leaching. This was probably due to the insufficiency of basic material.
7. The phosphorus content of the corn plants increased with increased growth.
8. Leaching decreased the calcium and increased the nitrogen content of the plants.
9. Leaching increased the water content of the plants, as a result, undoubtedly of the lessened concentration of soluble salts in the soil medium.

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PLATE I

The Effect of Leaching and of the Form of Phosphate and Nitrogen Salt on the Growth of Corn

Pot no	*Treatment*
35	Check
37	Acid phosphate, $NaNO_3$, unleached
45	Acid phosphate, $NaNO_3$, leached
39	Rock phosphate, $NaNO_3$, unleached
47	Rock phosphate, $NaNO_3$, leached
41	Acid phosphate, NH_4NO_3, unleached
49	Acid phosphate, NH_4NO_3, leached
43	Rock phosphate, NH_4NO_3, unleached
51	Rock phosphate, NH_4NO_3, leached

EFFECT OF LEACHING ON ROCK PHOSPHATE
F C BAUER

PLATE 1

PLATE 2

THE EFFECT OF LEACHING ON THE AVAILABILITY OF ROCK PHOSPHATE TO CORN

Pot no	*Treatment*
1	Leached (pot 45)
2	Unleached (pot 39)

THE LIME REQUIREMENT OF SOILS ACCORDING TO THE VEITCH METHOD, COMPARED WITH THE HYDROGEN-ION CONCENTRATION OF THE SOIL EXTRACT

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The measurement of the hydrogen-ion concentration of solutions has recently come into quite general favor. When the proper apparatus has been set up or color standards prepared, it is a rapid method and gives very satisfactory results.

Thus far, the method has not been widely used by soil investigators. Gillespie (2) has reported electrometric and colorimetric results on 22 samples of soil from different parts of the country. Gillespie and Hurst (3, 4) have used this method to determine the reaction of potato soils in relation to the presence or absence of the scab fungus and it is quite possible that it may have a wide application in this connection.

Martin (8) has used it for the same purpose and also for determining the relative hydrogen-ion concentration of extracts from soils to which varying amounts of sulfur were applied.

It is not quite clear to what extent the method may be employed in the determination of the lime requirement of soils. In this connection, Gillespie and Hurst say:

> It will be recalled that the intensity of acidity is measured by the hydrogen-ion concentration, and that it bears in general no simple or direct relation to the quantity of acid present. It has been shown many times that the hydrogen-ion concentration possesses a greater significance in biochemical processes than the quantity or concentration of acid substance. It is therefore entirely possible that certain problems of soil fertility, especially those relating to the necessity or desirability of liming for any specific crop, which have not as yet been solved by means of determinations of "lime requirement," may be solved by measurements of hydrogen-ion concentration.

Sharp and Hoagland (11, 12) have made determinations of hydrogen-ion concentrations of suspensions of unground soil, of soil ground to pass a 200-mesh screen and of heated soil. They also used the electrometric titration method in an attempt to determine the lime requirement by adding to the soil suspension, standard calcium hydroxide until a definite alkaline solution was obtained. This work they supplemented by pot and beaker studies.

Kappen and Zapfe (6) determined the titratable acidity and the hydrogen-ion concentration in extracts of peat soils and of peat and humus-forming plants.

Hoagland and Sharp (5) have studied the hydrogen-ion concentration of soil suspensions under various partial pressures of carbon dioxide.

Plummer (9) used the hydrogen electrode in determining the reaction of a large number of untreated soils in suspension. He also determined the reaction of soils that had received different fertilizing materials.

Rice and Osugi (10) heated soil with a solution of cane sugar, determined the quantity of invert sugar, and in another portion of the filtrate determined the hydrogen-ion concentration by the color method of Sörensen.

In some recent work in connection with the lime experiments which are being conducted at this station, the authors had an opportunity to compare lime requirement data secured by the Veitch method with the hydrogen-ion concentration of water extracts from the same soils. We do not maintain that results obtained by the one method may be directly interpreted in terms of the other, but feel that the data secured are of sufficient importance to warrant publication. The soils in question were taken from plots which are laid out in four different 5-year rotation systems, with seven plots for each system. For a full description of these plots and the lime treatment see Lipman and Blair (7). The lime treatment for each rotation system is as follows:

Plot 1	check (no lime)
Plot 2	½ ton calcium limestone per acre
Plot 3	1 ton calcium limestone per acre
Plot 4	2 tons calcium limestone per acre
Plot 5	½ ton magnesian limestone per acre
Plot 6	1 ton magnesian limestone per acre
Plot 7	2 tons magnesian limestone per acre

The soil is a Sassafras loam inclining to the gravelly phase. Previous to 1908, the land had been neglected for a number of years. Limestone was applied in accordance with the above plan, first in the spring of 1908 and again in the spring of 1913 and of 1918.

The four rotations as carried out during the period of 1913 to 1917 were as follows:

	ROTATION 1, PLOTS 21–27	ROTATION 2, PLOTS 28–34	ROTATION 3, PLOTS 35–41	ROTATION 4 PLOTS 42–48
1913	Corn (rye, vetch, crimson clover)	Corn (rye, vetch, crimson clover)	Corn (rye, vetch, crimson clover)	Corn (rye, vetch, crimson clover)
1914	Oats (soybeans and cowpeas)	Potatoes	Potatoes (rye)	Oats and peas, millet
1915	Wheat	Rye	Tomatoes (rye, vetch, crimson clover)	Rye and vetch, rape
1916	Timothy and clover	Timothy and clover	Lima beans (rye, vetch, crimson clover)	Rye, cowpeas (rye)
1917	Timothy and clover	Timothy and clover	Cucumbers (rye and vetch)	Oats and peas, cowpeas

For the period of 1908 to 1912 the crops were practically the same as for 1913 to 1917.

TABLE 1

Lime requirement and hydrogen-ion concentration of soils from plots that have received different quantities of lime

PLOT NUMBER	SPECIAL TREATMENT	LIME (CaO) REQUIREMENT PER 2,000 000 POUNDS OF SOIL		pH VALUES, 1919
		1913	1919	
	Rotation 1 General farm crops			
		lbs	*lbs*	
21	Nothing	1,200	1,500	5 4
22	0 5 ton $CaCO_3$ per acre	1,000	500	6 1
23	1 0 ton $CaCO_3$ per acre	600	400	6 7
24	2 0 tons $CaCO_3$ per acre	000	000	7 2
25	0 5 ton $CaCO_3MgCO_3$ per acre	600	400	6 0
26	1 0 ton $CaCO_3MgCO_3$ per acre	700	100	6 5
27	2 0 tons $CaCO_3MgCO_3$ per acre	000	000	7 0
	Rotation 2 General farm crops			
28	Nothing	800	1,200	5 8
29	0 5 ton $CaCO_3$ per acre	800	400	6 0
30	1 0 ton $CaCO_3$ per acre	600	000	6 5
31	2 0 tons $CaCO_3$ per acre	100	000	7 1
32	0 5 ton $CaCO_3MgCO_3$ per acre	700	800	6 3
33	1 0 ton $CaCO_3MgCO_3$ per acre	400	000	6 7
34	2 0 tons $CaCO_3MgCO_3$ per acre	300	000	6 9
	Rotation 3 Corn, potatoes, market garden crops			
35	Nothing	1,100	1,600	5 5
36	0 5 ton $CaCO_3$ per acre	800	1,200	6 1
37	1 0 ton $CaCO_3$ per acre	600	200	6 3
38	2 0 tons $CaCO_3$ per acre	400	000	7 1
39	0 5 ton $CaCO_3MgCO_3$ per acre	1,100	800	6 2
40	1 0 ton $CaCO_3MgCO_3$ per acre	700	600	6 5
41	2 0 tons $CaCO_3MgCO_3$ per acre	500	000	6 9
	Rotation 4 Forage crops			
42	Nothing	1,200	800	5 4
43	0 5 ton $CaCO_3$ per acre	1,100	600	6 0
44	1 0 ton $CaCO_3$ per acre	700	400	6 3
45	2 0 tons $CaCO_3$ per acre	600	000	7 1
46	0 5 ton $CaCO_3MgCO_3$ per acre	1,100	400	6 2
47	1 0 ton $CaCO_3MgCO_3$ per acre	500	200	6 4
48	2 0 tons $CaCO_3MgCO_3$ per acre	300	000	6 9

The samples of soil were collected during the fall of 1919 so that something over a year had elapsed since the last application of limestone. In collecting the samples, nine borings were made at different points in the

$\frac{1}{10}$-acre plot and these were then thoroughly mixed; brought to the laboratory where they were dried and passed through a 1-mm. sieve preparatory to analysis. Lime-requirement determinations were made by the Veitch method and at the same time, the hydrogen-ion concentration of the soil extract was determined by using the phenolsulfonphthalein series of indicators suggested by Clark and Lubs (1). The soil extract was prepared by shaking 15 gm. of soil with 30 cc. of distilled water and centrifuging until a clear solution was obtained. The results from the two methods are shown in table 1. From this it will be noted that almost without exception the lime requirement

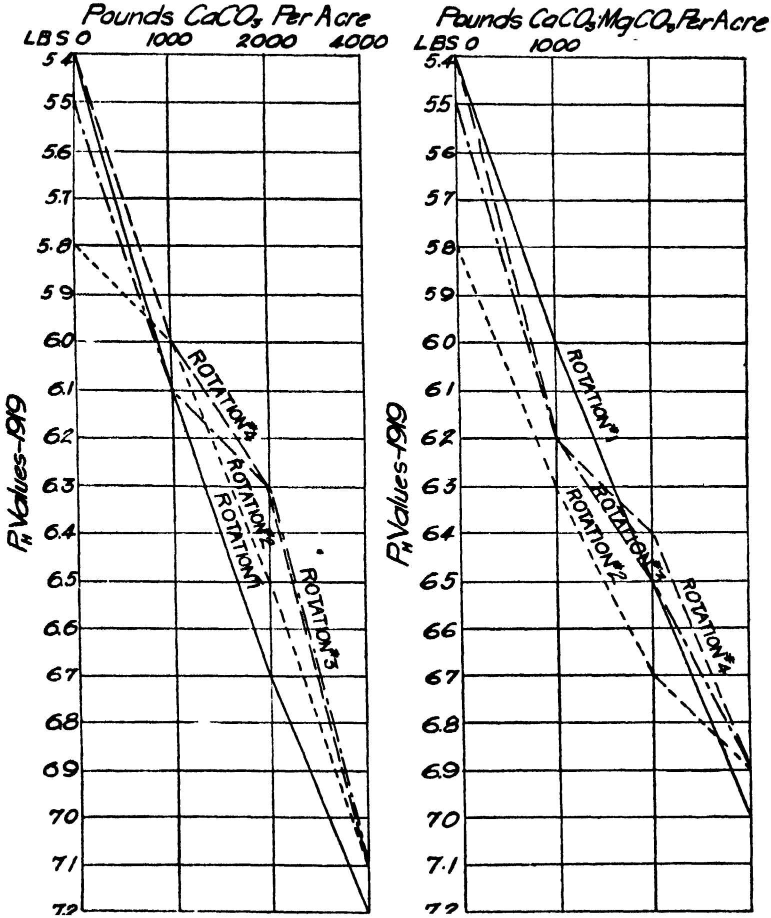

FIG. 1. DIAGRAM OF pH VALUES

decreased as the amount of limestone applied was increased. However, the decreases are not necessarily proportional to the amount of limestone applied.

With an application of 1000 pounds of $CaCO_3$ the range in lime requirement for rotations 1, 2 and 4 is from 600 to 400 pounds per acre, and the hydrogen-ion exponent expressed as pH, using the terminology of Sörensen, is about 6.1. The corresponding sample from rotation 3 shows a requirement of 1200 pounds, and a pH value of 6.1. It will be noted that the pH value in these two cases is practically the same, and in this connection it is well to remember that the hydrogen-ion concentration is a measure of the intensity of acidity and not of the titratable acidity. With 1000 pounds of the magnesian limestone, the lime requirement varies from 800 to 400 pounds per acre and the hydrogen-ion exponent from 6.0 to 6.3. With 2000 pounds of the limestone, both calcium and magnesium, the lime requirement varies from 600 pounds to an alkaline reaction, and the hydrogen-ion exponent from 6.3 to 6.7.

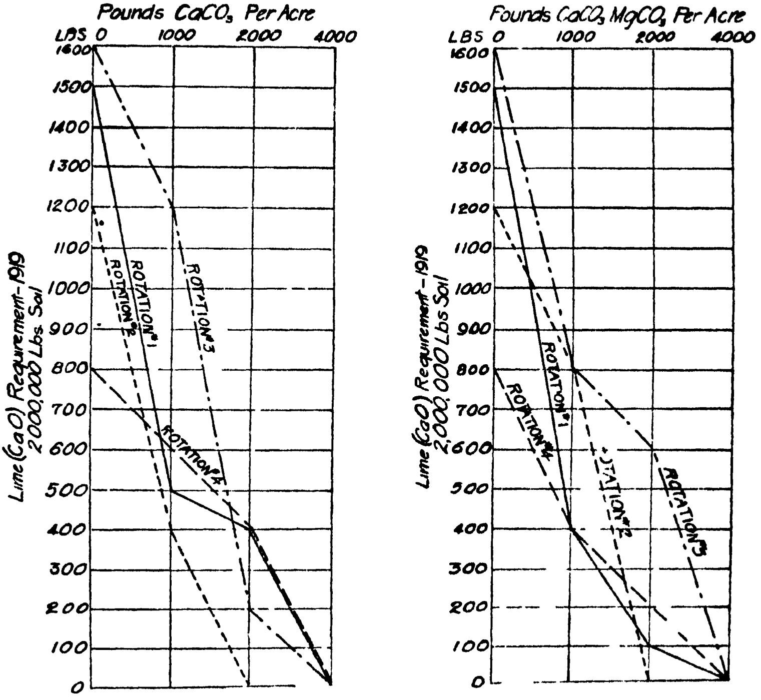

FIG. 2. DIAGRAM OF LIME REQUIREMENTS ACCORDING TO THE VEITCH METHOD

With the 4000-pound application of limestone, the Veitch method gives the alkaline reaction in all cases (this method, of course, can not show any distinction in degree of alkalinity), and the hydrogen-ion exponent varies from 7.1 to 7.2 with the calcium limestone and from 6.9 to 7.0 with the magnesian limestone.

The pH values over 6.7 require no lime.

Between pH = 6.3 and 6.7, 800 pounds is the maximum requirement.

Between pH = 6.0 and 6.3, 1200 pounds is the maximum requirement.

Between pH = 5.4 and 6.0, 1600 pounds is the maximum requirement.

Although the lime requirement may fall much below the maximum given here, it would be a distinct advantage to know the maximum requirement at a certain hydrogen-ion concentration.

Further work must be done on a variety of soil types before any general conclusions can be drawn. Tests have shown that soils containing high percentages of organic matter, such as muck soils, do not show any direct relationship between the hydrogen-ion concentration of the soil extract, and the lime requirement by the Veitch method. In such cases the lime requirement is much higher than would be expected from the pH values. This is no doubt due to "buffer" materials which are present in such soils.

For the check plots where no lime was added the lime requirement varies in the four rotations from 1600 to 800 pounds and the pH values from 5.4 to 5.8.

The differences referred to are more clearly brought out in the accompanying graphs.

A study of the pH values shown in table 1 leads to the following suggestions.

SUGGESTIONS

1. For the samples tested, there appears to be a fairly close correlation between the hydrogen-ion concentration of the soil extract and the lime requirement as determined by the Veitch method.
2. Certain inconsistencies appear which may be due to the inaccuracies of the Veitch method or to the lack of uniformity in the samples, or to the influence of "buffer" substances.
3. With normal soils, a determination of the hydrogen-ion concentration of the soil solution may give one some idea of the amount of lime water required by the Veitch method, and thus considerably shorten this method.
4. Of the soils under consideration, those which have a hydrogen-ion concentration of about pH = 6.7 or over, are alkaline by the Veitch method.
5. With further studies along this line, it may be possible, with normal soils, to assign a fairly definite lime requirement to a given hydrogen-ion concentration, so that in many cases at least a determination of the hydrogen-ion concentration would make a lime-requiremcmt determination unnecessary. If such should prove to be the case, the gain in time will be an important item.

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HYDROGEN-ION CONCENTRATION MEASUREMENTS OF SOILS IN CONNECTION WITH THEIR "LIME-REQUIREMENTS"

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The work on hydrogen-ion concentration measurements of the soils is still in its infancy. Notwithstanding the importance of such measurements in soil-fertility problems very little has been done in this field. In biochemical work hydrogen-ion concentration measurements are of enormous significance as pointed out by Michaelis (4) and Clark and Lubs (1). Sharp and Hoagland (5) report on the acidity and absorption in soils as measured by the hydrogen electrode. Gillespie and Hurst (3) call attention to the fact that it is possible to exploit hydrogen-ion concentration studies in problems of soil fertility, especially those connected with "lime-requirements" in general, and specific crops in particular. The soil may be looked upon in a way, as a culture medium for microorganisms which have a direct bearing upon soil fertility. Reaction studies of the soil are being conducted at the New Jersey station, and this paper is simply a report on some work bearing indirectly on the subject.

The question of how much lime should be applied to a particular field for a certain crop has not as yet been answered satisfactorily in the light of studies of the intensity of the acidity, as measured by the hydrogen-ion concentration method. We must, for the present, use the old Veitch method and its numerous modifications, with all their deficiencies. The determination of the lime-requirement by the Veitch method requires a preliminary investigation on the approximate acidity of a given soil; this is followed by the trial method of determining the titrable acidity. In this method and many of its modifications the reaction best suited to the particular crop is not taken into consideration, and in this respect they are of no value.

An attempt was made to see whether there is a correlation between the lime requirement as determined by the Veitch method and the curve of pH values resulting from the lime-water treatment.

Table 1 gives a description of the soils used in the work.

The hydrogen-ion concentration of these soils, as expressed in pH values, was determined by the colorimetric method, using the phenolsulfonephthalein indicator series as described by Clark and Lubs (1). The results appear in Table 2.

Those soils that approached the neutrality point were treated with little lime-water; this did not, however, give any idea as to how much lime-water

TABLE 1

Description of soils used

LABORATORY NUMBER	TYPE OF SOIL	TREATMENT	REMARKS
1	Mud bottom; mostly vegetable residues		Good cranberry-producing soil
2	Savannah bottom; mostly sand; little organic matter		Good cranberry-producing soil
3	Iron-ore bottom; mostly vegetable residues		Good cranberry-producing soil
4	Mud bottom; sandy soil with much organic matter	4000 pounds of limestone per acre applied in 1914.	First half-inch surface soil
5	Same as 4, but less organic matter		Second half-inch surface soil
6	Still less organic matter		From first to second inch depth
7	Mostly sand		From second to fourth inch depth
	Soil 8, 9, 10, 11, is a check on 4, 5, 6, 7; no lime applied		
12	Mud bottom; much organic matter, resembles soil 8	Concentrated sulfuric acid applied to kill weeds	No cranberries grown
13	Savannah bottom; mostly sand	4000 pounds of limestone per acre applied in 1918	
14	Mud bottom; sandy soil with much organic matter		
15	Iron-ore bottom; very little organic matter		
16	Mud bottom; same as 14		Check on soil 14
17	Mostly sand; some undecayed vegetable matter	Sulfuric acid applied to kill weeds, followed by limestone to correct acidity	No cranberries
18	Same as 17	Acid applied, but no lime	No cranberries

TABLE 1—*Concluded*

LABORATORY NUMBER	TYPE OF SOIL	TREATMENT	REMARKS
19	Mostly sand, but considerable vegetable matter	Sulfuric acid applied first, then rock phosphate	No cranberries
20	Same as 19	Acid applied but no rock phosphate	No cranberries
21	Mostly vegetable residues	No treatment	First half-inch of surface soil
22	Same as 21 but a little sand in addition	No treatment	Second half inch of soil
23	Same as 22 with still more sand; but mostly vegetable residue	No treatment	From first to second inch depth
24	Same as 23 with somewhat more sand	No treatment	From second to fourth inch depth

TABLE 2

Hydrogen-ion concentration of soils compared with lime requirement according to the Veitch method

LABORATORY NUMBER	pH VALUE BEFORE TREATMENT	LIME-WATER REQUIRED FOR NEUTRALIZATION AS DETERMINED BY THE VEITCH METHOD (1 CC OF LIME WATER = 0 009607 GM CaO).	pH VALUE BEFORE EVAPORATING SAMPLE FOR THE LIME REQUIREMENT DETERMINATION BY THE VEITCH METHOD	pH VALUE AFTER EVAPORATION
		cc.		
1	5 2	50 00	6 9	8 2
2	4 8	25 00	7.2	8 0
3	5 2	49 00	7.0	8.0
4	7 0	1 00	6 8	7.9
5	6 4	5 00	6 8	8.0
6	6 2	10 00	6 6	8 0
7	5.8	10 00	6 8	8 1
8	5 0	48 00	7 0	7 9
9	5 4	26 00	6 9	7.9
10	5 0	40 00	7.0	8.1
11	5.4	15 00	7.1	8.2
12	5 0	52 00	6 8	8.0
13	5 4	22.00	7.0	8.2
14	6 0	30.00		8 0
15	6 2	8 00	6.7	8.2
16	4 4	40 00	6 6	8.0
17	4 6	45 00	7.1	8.2
18	4.2	60 00	6.8	8.0
19	4.4	45.00	7.0	8.2
20	4 2	65 00	6.6	8.0
21	4 0	78 00	6 8	8.0
22	4.0	65 00	6 9	8.2
23	4.1	60.00	6 9	8.2
24	3.9	50 00	6 9	8.2

was to be used. The procedure, therefore, consisted of the regular Veitch method. With every trial, on a fresh addition of lime, the pH values were determined before and after evaporation. In the case of the sandy soil samples, which are rather poor in organic matter, less lime-water was required to reach the maximum (the point where the solution turns pink after evaporating the filtrate, in the Veitch procedure) independently by the initial pH value than with those that had a higher humus content. This can easily be seen in table 2. Samples 1 and 3, soils consisting chiefly of organic material, required 49 and 50 cc. of lime-water, although the original pH value was 5.2 in each case, while sample 2, which was of a sandy nature, required only 25 cc. with an original pH value of 4.8, i.e., a more acid reaction than either samples 1 or 3. The same is noticeable in the case of the other samples.

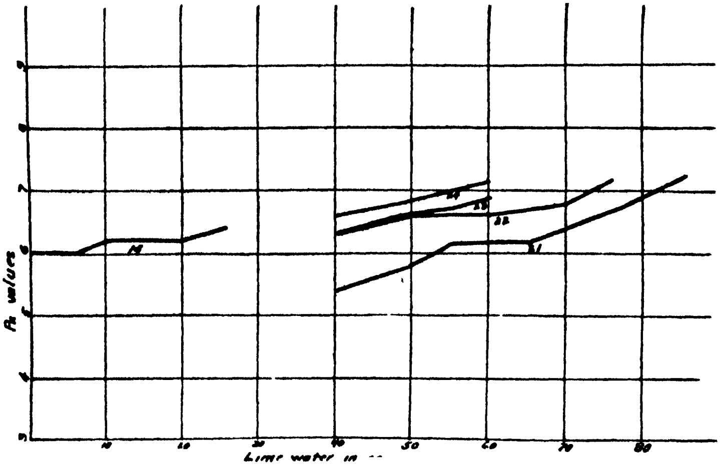

FIG 1. CURVES OF HYDROGEN-ION CONCENTRATION OF SAMPLES 14, 21, 22, 23 AND 24

The explanation for this is apparent. The sandy soil has no buffers and the addition of lime-water increases the concentration of the hydroxyl-ions, which combine with the hydrogen-ions, forming water; in the case of the soils with a large amount of organic substance, the amount of buffer is much larger and thus prevents the suppression of the hydrogen ions.

The amount of buffer in soils with an abundance of plant residues will differ with each soil; the slightest change in the organic substances will produce a different amount of complex organic compounds with different degrees of buffer action; the amount of buffer can be measured when the hydroxylions reach a point where the buffer has no more effect and the curve begins to rise gradually; this point is the end-point of the buffer action. The curves of samples 14, 20 and 21 illustrate the effect of buffers.

It seems to the author that in the case of sandy soil with little organic matter, an adjustment of the reaction to neutrality, or any point desired, may be accomlished very conveniently by the hydrogen-ion concentration method in a very short time, avoiding the tedious procedure of the Veitch method The curves of samples 13, 15, 18, 23 and 24 show the possibilities of adjusting the soil reaction in sandy soils. It is of interest to note the relation of the curves of samples 21, 22, 23 and 24. They are from the same soil of different depths, as pointed out in table 1. It is characteristic that the surface soil with the plant residues has an irregular curve, and as we approach the lower depths the tendency of the curve is to become regular. The same kind of a curve would be produced by samples 8, 9, 10 and 11. Looking over the pH values before and after evaporation, we notice that if we get a pH value between 6.6 and 6.8 before evaporation we are certain that we have reached the point of the "lime requirement." By such a procedure we may save more than half the time which the evaporations in the Veitch method take.

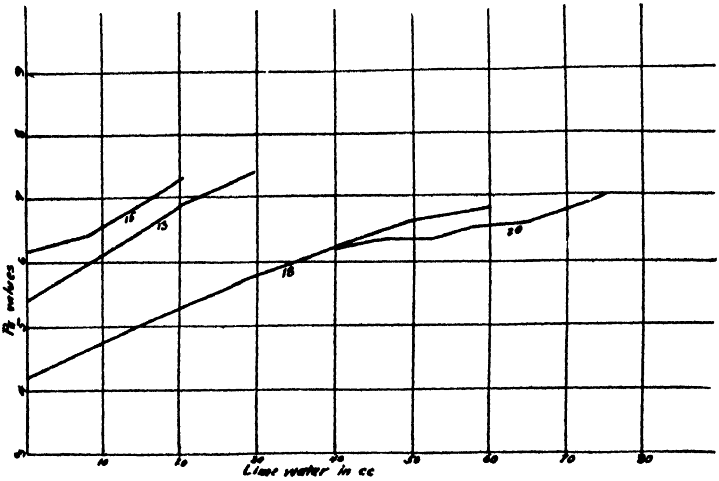

FIG. 2. CURVES OF HYDROGEN-ION CONCENTRATION OF SAMPLES 13, 15, 18 AND 20

As was stated in the earlier part of this article, this is not a study of the hydrogen-ion concentration measurements of soils. Incidentally, it points toward a method for the determination and adjustment of the soil reaction in sandy soils and possibly even in soils with a high organic-matter content. The author feels that more elaborate experimentation in this line is essential, in order to establish the proposed scheme.

The author takes this opportunity to thank Dr. Selman A. Waksman for reading the manuscript and offering helpful criticism.

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THE RELATIVE ABSORPTION BY SOIL OF SODIUM CARBONATE AND SODIUM CHLORIDE

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A number of papers have been published recently which describe investigations of the effect upon plant growth of "alkali" salts when added to the soil and in which conclusions as to the relative toxicity of the different salts are based upon the quantity of salt added (5, 7, 12).[1] The disadvantage of this method of interpreting the results is that it does not take into account differences in the proportion of the solute which is withdrawn from the solution when different salts are brought into contact with a soil. This source of error was noted several years ago by Mr. W. H. Heileman, formerly of the Bureau of Soils, United States Department of Agriculture, in the course of experiments with seedling plants grown in soils to which salts of sodium had been added. He observed that under these conditions sodium carbonate appeared to be much less toxic in comparison with sodium chloride than had been indicated by numerous observations upon plants growing in natural alkali soils and by the results of water culture experiments.[2]

It has been shown by Headley, Curtis and Scofield (8) that when sodium carbonate is added to a soil, upon analysis of the soil extract after several weeks, the quantity recovered is much less than the quantity which had been added, even when allowance is made for the increase in the content of bicarbonates which has taken place. In the case of sand, to which had been added solutions of sodium carbonate in eight concentrations ranging from 0.05 to 0.40 per cent, the quantity recovered of sodium carbonate plus excess of sodium bicarbonate reckoned as carbonate averaged 69.0 ± 2.9 per cent. In the case of a loam soil to which had been added solution of sodium carbonate in 10 concentrations ranging from 0.05 to 0.60 per cent, the quantity recovered averaged only 26.0 ± 1 7 per cent.[3] In other words the sand had absorbed 31 per cent and the loam had absorbed 74 per cent of the sodium carbonate which had been added to the soil.

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[1] The readiness with which sodium carbonate is absorbed, and the necessity of considering this factor in relation to the toxicity of soils to which this salt has been added, are recognized in other publications by Harris and Pittman (6, 11).

[2] Thus Kearney and Cameron (9, p 19, 24, 33, 36) found that both in pure solution and in the presence of an excess of calcium carbonate and of calcium sulfate, sodium carbonate was much more toxic to the roots of white lupine than was sodium chloride. Similar results with various crop plants were obtained by Kearney and Harter (10).

[3] These percentages have been computed from data given in table 3, p. 863 of the publication cited.

Very different results with sodium chloride were obtained by the same investigators. This salt was added to a loam soil in six concentrations of solution ranging from 0.05 to 0.60 per cent and when the soil extract was analyzed several weeks later the quantity of sodium chloride recovered averaged 84.0 ± 1.5 per cent.[4] In a parallel experiment with the same soil to which had been added sodium carbonate solutions in eight concentrations ranging from 0.05 to 0.40 per cent, the quantity recovered of sodium carbonate, plus excess sodium bicarbonate reckoned as the carbonate, averaged only 23.5 ± 2.3 per cent.[5] In other words, the loam soil had absorbed 76.5 per cent of the sodium carbonate and only 16 per cent of the sodium chloride which had been added.

A further source of error, in experiments dealing with the relative toxicity of sodium carbonate, when conclusions are based solely upon the quantity of salt which has been added to the soil, lies in leaving out of account the reaction which takes place with carbon dioxide, resulting in the formation of the bicarbonate at the expense of the normal carbonate. It was demonstrated by Cameron and Briggs (3) that in solutions of sodium carbonate at a temperature of 25°C. and in concentrations ranging from 0.2 to 0.6 per cent, approximately half of the normal carbonate is replaced by the bicarbonate. In the above cited publication by Headley, Curtis and Scofield, evidence is given that a similar reaction occurs when sodium carbonate is added to soil, a large proportion of the salt being recovered in the form of bicarbonate. Sodium bicarbonate is less toxic than the normal carbonate, as was shown by the results of an experiment performed by Kearney and Cameron (9, p. 19 and 20; see also ref. 10) in which roots of the white lupine were exposed to solutions of sodium bicarbonate wherein the formation of the normal carbonate was prevented by the presence of an excess of carbon dioxide. Therefore when sodium carbonate is added to a soil, not only is the total concentration of the soil solution much smaller than would be assumed from the quantity of salt added, but much of the salt which remains in the soil solution is in the form of the less toxic bicarbonate.[6]

RELATIVE ABSORPTION AS INDICATED BY ELECTRICAL RESISTANCE

A comparison of different salts of sodium in respect to the relative degree of absorption in a soil, as measured by the electrical resistance of the system, was made by Davis and Bryan (4), who, however, apparently made their readings immediately after the solutions were added to the soil. In order to test adequately the usefulness of the electrical resistance method for determining differences in absorption of different salts by a soil, more time should be allowed for the system to reach an equilibrium. This has been done in an experiment performed by the writer, which is described below.

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[4] As computed from data given in table 5, p. 867.

[5] As computed from data given in table 2, p. 861.

[6] It might be thought that owing to the unstable equilibrium between the carbonate and bicarbonate, the relative toxicity of the two salts is of no practical importance. As a matter of fact, however, bicarbonates are sometimes present in large quantity in soils which do not give the hydroxyl reaction with phenolphthalein.

Solutions of sodium chloride and sodium carbonate, of concentrations ranging from 0.05 to 1.00 per cent, were added to air-dry sand in sufficient quantity to supersaturate it slightly.

The sand used had a moisture equivalent[7] of 2.4 per cent, indicating a moisture-holding capacity of 25 per cent and the quantity of solution added was 26 per cent of the dry weight of the sand. This sand was relatively free from readily soluble material, as is indicated by the fact that when saturated with distilled water and allowed to stand 24 hours, its electrical resistance was about 2500 ohms. When the sand was moistened with the solution the mixture was thoroughly stirred and was allowed to stand for 24 hours, when it was again stirred. The cup of the electric bridge was then filled with the wet sand and the electrical resistance of the latter was determined and was corrected to a temperature of 60°F.

TABLE 1

Electrical resistances at 60°F. of the free solutions when the cup is filled to 40 per cent and to 100 per cent of its capacity (20 cc. and 50 cc., respectively)

CONCENTRATION OF SOLUTION	SODIUM CARBONATE			SODIUM CHLORIDE		
	Resistance of solution		Ratio of resistance of 20 cc to that of 50 cc	Resistance of solution		Ratio of resistance of 20 cc to that of 50 cc
	20 cc	50 cc		20 cc	50 cc.	
per cent	*ohms*	*ohms*		*ohms*	*ohms*	
1.00	63	22	2.86	53	19	2.79
0.80	75	28	2.68	65	24	2.71
0.60	98	34	2.88	89	32	2.78
0.40	135	49	2.75	126	46	2.74
0.20	246	90	2.73	240	85	2.82
0.10	500	167	2.99	467	171	2.73
0.05	872	328	2.65	872	323	2.70
	Average ratio 2.79 ± 0.03			Average ratio 2.75 ± 0.01		

The electrical resistances of the different concentrations of the free solution of each salt also were determined, both with the bridge cup full (50 cc.) and with only 20 cc. of solution in the cup, the latter corresponding to the moisture content of the cup when filled with the wet sand.

The resistances of 20 cc. and of 50 cc. of each concentration of solution of the two salts are stated in table 1, which also gives for each concentration the ratio of the two resistances. It is evident that, for the range of concentrations used, this ratio is practically a constant. It is also evident that with an equal concentration and volume of solution the resistances of the two salts differ only slightly.

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[7] As determined by the centrifugal method (1). A formula for computing the moisture-holding capacity from the moisture equivalent is given by Briggs and Shantz (2).

In table 2 are given, for each concentration of each salt, the resistance of the saturated sand (containing 20 cc. of the solution), the resistance of 20 cc. of the free solution and the ratio of the first to the second resistance. The ratios are graphically expressed in figure 1.

If the presence of the sand had had no effect upon the resistance of the solution in contact with it, the ratio of the two resistances for each concentration of each salt should have been 1.0, since the quantity and the original concentration of solution were the same in both cases. The degree to which the ratio exceeds unity therefore indicates the degree to which the resistance of the solution in contact with the sand has been increased by withdrawal of a portion of the solute. It is evident that in the case of sodium carbonate much more of the solute has been withdrawn than in the case of sodium chloride, the dilution due to contact with the sand having increased the resistance, for the several concentrations, nearly twice as much in the former case as in the latter.

TABLE 2

Resistances at 60°F. of the saturated sand (cup full) and of 20 cc. of the corresponding free solution

CONCENTRATION OF SOLUTION	SODIUM CARBONATE			SODIUM CHLORIDE		
	Resistance of		Ratio of resistance of sand to solution	Resistance of		Ratio of resistance of sand to solution
	Saturated sand	Solution (20 cc.)		Saturated sand	Solution (20 cc.)	
per cent	*ohms*	*ohms*		*ohms*	*ohms*	
1.00	146	63	2.3	77	53	1.5
0.80	198	75	2.6	90	65	1.4
0.60	234	98	2.4	116	89	1.3
0.40	416	135	3.1	180	126	1.4
0.20	765	246	3.1	317	240	1.3
0.10	1159	500	2.3	594	467	1.3
0.05	1539	872	1.8	1014	872	1.2
0	2488*			2488*		
	Average ratio 2.5 ± 0.12			Average ratio 1.3 ± 0.02		

* Control, sand saturated with distilled water.

A glance at figure 1 shows that the curves representing the ratios of the two resistances differ greatly for the two salts, that for sodium chloride being much flatter than the curve for sodium carbonate, which shows a conspicuous maximum at concentrations of 0.20 and 0.40 per cent. Discussion of this difference, the significance of which could be ascertained only by repeated determinations, is beside the purpose of the present article, since the data given sufficiently answer the question whether sodium carbonate is withdrawn from solution in greater proportion than sodium chloride, when in contact with soil.

It should be noted that the resistances of sand saturated with solutions of corresponding concentration of sodium carbonate and of sodium chloride, as given in the publication of Davis and Bryan (4, p. 13, table 1), are much

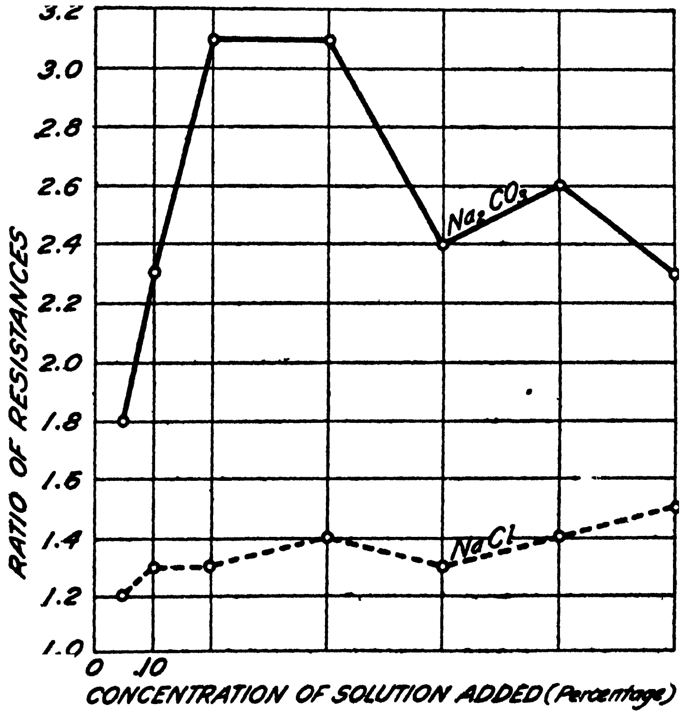

FIG. 1. RATIO OF RESISTANCE OF SAND PLUS 20 CC. OF SOLUTION TO THAT OF 20 CC. OF FREE SOLUTION

lower than the resistances in sand obtained by the writer, and also show little difference as between the two salts. The probable explanation is that Davis and Bryan determined the resistance immediately after adding the solution to the soil, while in the writer's experiment the readings were made after solution and soil had remaired in contact during 24 hours.

Inspection of table 2 shows that when this amount of time is allowed, sand to which sodium carbonate has been added at concentrations of 0.10 to 1.00 per cent, gives a resistance about double that of sand to which like concentrations of sodium chloride have been added. Roughly speaking, the soil solution in the latter case is about twice as concentrated as in the former, yet if unaware of how differently the two salts are absorbed by the sand, one might assume that the soil solutions, like the original solutions, were of equal concentration in both cases.

In order to obtain some idea of the actual concentration, of the solution present in the sand to which sodium carbonate had been added 24 hours previously, a curve was plotted for the electrical resistances of 20 cc. of the free solution at the several concentrations which had been added. The curve was extended so as to include resistances as high as those of the sand to which the two most dilute solutions had been added, by determining the resistances of 20 cc. each of 0.04 per cent and 0.03 per cent sodium carbonate in free solution, the resistances obtained for these concentrations having been 1120 and 1580 ohms, respectively. By reading on the curve the concentration corresponding to the electrical resistance of the saturated sand, an approximate idea was obtained of the concentration of the solution present in the latter. For the 6 concentrations 0.10 to 1.00 per cent, the concentration of the solution present in the sand as thus estimated averaged 37 per cent (range 34 to 40 per cent) of that which had been added, indicating that approximately 63 per cent of the salt had been withdrawn from the solution when in contact with the sand. A similar calculation in the case of sodium chloride indicated an average absorption of only 23 per cent of the salt which had been added.

CONCLUSIONS

When equal volumes of solution of equal concentration of sodium carbonate and of sodium chloride are added to sand and the solution and soil are allowed to remain in contact during several hours, the electrical resistance of the sand to which sodium carbonate has been added is much higher than that of the sand to which sodium chloride has been added.

Since the greater resistance in the case of sodium carbonate must be due to proportionately greater withdrawal of the solute by the sand, it follows that plants growing in soils to which equal quantities of the two salts have been added are in contact with soil solutions of very unequal concentration.

As a result of overlooking this factor, as well as the reaction which takes place in solutions of sodium carbonate resulting in the formation of the less harmful bicarbonate, certain investigators of the effects of "alkali" salts upon plant growth have concluded that sodium carbonate is less toxic than sodium chloride. Observations upon plants growing in natural alkali soils and experiments with seedlings exposed to pure solutions of these salts have shown the contrary to be true.

The results of the experiment described in this paper indicate that the electrical bridge affords a convenient means for determining the degree to which different salts are withdrawn from a solution which has been added to a soil. In the case of sodium carbonate and sodium chloride, equivalent solutions of which (at the concentrations ordinarily encountered in alkali soils) do not differ greatly in electrical resistance, the bridge method permits direct comparison of the concentration of the solution in soils to which these salts have been added.

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SYMBIOTIC NITROGEN FIXATION AS INFLUENCED BY THE NITROGEN IN THE SOIL[1]

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I. INTRODUCTION

Of the important elements necessary for growing plants, nitrogen is the one that presents our most serious problem and is most apt to be deficient. Early studies on the different forms in which nitrogen may serve for plant growth seemed to have it definitely settled that only combined nitrogen could be used by plants (6, 30). Atwater (2), however, showed that legumes, quite contrary to earlier beliefs, were able indirectly to utilize the elementary nitrogen. This prompted many researches and opened many discussions, which soon established the fact that certain plants, belonging to the *Leguminosae*, were able to obtain the gaseous nitrogen of the air through the action of bacteria living in the nodules on their roots.

When the fact became known that legumes are able to use the nitrogen of the air through a mutually beneficial relationship with bacteria, numerous studies of these plants were undertaken to determine the manner in which they take nitrogen from the air and incorporate or fix it in their tissues.

This process of "symbiotic nitrogen fixation," as it has been named, has taken on considerable significance in the attempt to maintain the nitrogen supply for plant growth. The fact that it offers a means of utilizing the unlimited supply of nitrogen of the air in place of the costly nitrogenous fertilizers, has served as an incentive to study this process and the factors which influence its highest development. Any information giving a clearer understanding of the process of symbiotic nitrogen fixation may be justified as contributing to the large agricultural problem of maintaining the supply of nitrogen in the soil in sufficient amounts to insure maximum crop production. The following research is a contribution to the process of nitrogen fixation by legumes as influenced by the amount of nitrogen present in the soil in both the organic and inorganic forms.

[1] A thesis submitted to the Faculty of the Graduate School of the University of Illinois in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, June, 1919.

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II. HISTORICAL

The literature on the subject of symbiotic nitrogen fixation is rather extensive and has been well collected in bibliographies by Jacobitz (27), by Burrill (7), and others, so that no extensive review on the subject is necessary. Only those papers dealing particularly with this process as influenced by the nitrogen content of the soil will be considered.

General statements are common in saying that the legume fulfills its needs for nitrogen from the soil and later resorts to the supply in the air. Conn (8) makes the statement that "legumes appear to prefer taking their nitrogenous material directly from the nitrogenous foods in the soil when these are present in abundance. But if the soil does not furnish the proper nitrogen, then recourse is had to atmospheric nitrogen, through the agency of tubercle organisms." Van Slyke (46) ventures a similar opinion in which he says, "when supplied with available nitrogen compounds, the bacteria fail to make use of atmospheric nitrogen." Hopkins (25, p. 217) agrees in substance with this. "Clover and other legumes," he says, "take available nitrogen from the soil in preference to the fixation of free nitrogen from the air, the latter being drawn upon only to supplement the soil's supply and thus balance the plant-food ration."

Early works show that nodules are present when the bacteria become established and when the plant uses atmospheric nitrogen. In much of the literature the nodules represent nitrogen fixation and unless this modification of the root is present, no use of gaseous nitrogen is believed to be taking place. The importance of nodules in nitrogen fixation was established early by Hellriegel and Wilfarth (19) as one of the fundamental facts when they say, "the nodules of the roots must not be considered as simply reservoirs of albuminoid substances, their relation to the assimilation of free nitrogen is that of cause to effect." In most works cited in the following discussion, the effects of the nitrogen in the soil as a cultural medium, are reported as favorable or unfavorable to the nodule production, and hence to the nitrogen fixation.

Moore (34) apparently does not agree with this general conception of the importance of nodules. He believes that it is possible for the bacteria to enter the roots and be of benefit without evincing their presence by such external evidence as nodules. He fails to believe that even if the absence of nodules might permit some nitrogen fixation, this would not prohibit the same performance in their presence.

Rautenberg and Kuhn (43) ventured perhaps the earliest statement concerning the relation of nitrogen fixation to the nitrogen in the medium for plant growth. In their work *Vicia faba*, growing in a nitrogen-free solution, developed numerous nodules, while in the presence of nitrates no nodules appeared. De Vries (10) obtained similar results while studying nodules as a storage for nitrogen. In the absence of nitrogen from the cultural solution, many nodules of normal structure were produced, but otherwise scarcely any developed.

Schindler (44) working with clovers, vetch, serradella, kidney vetch, and beans in water cultures concludes that, in general, solutions rich in nitrogen are less disposed toward giving nodules than those lacking in this element. These same plants grown in soil fertilized with compost or manure as compared with soils low in nitrogen gave larger and more numerous nodules in every case with the latter soil. Tschirch (45) in studying nodules, as a means of nitrogen storage, says that it is established that nodules grow more profusely in soils poor in nitrogen than in those rich in humus.

Vines (47) treated soils with 1 per cent potassium nitrate and found nodular development decreased, and also an indication that as the amount of nitrate diminished, the development of nodules became more marked. Similar depressive effects on nodule production by nitrates are reported also by Baszler (3) and by Laurent (28).

These studies on the influence of the nitrogen content of the soil on the nodule development, take this development as a measure of nitrogen fixation, but fail, however, to substantiate their contentions with careful chemical analyses to show that the total nitrogen in the plant and soil has truly increased.

Frank (13) studied conditions of the plant as influenced by inoculation and other factors. In using soils rich in humus as compared with those very poor in this respect, he was led to believe that when humus is present in sufficient amounts the bacteria are dispensable and serve with no benefit to the plant. Where humus is lacking the bacteria are active. This explains why legumes can be grown on sand when all minerals are supplied even when no humus is present.

Atwater (2) in growing peas in sand supplied with nutrient solutions containing varying amounts of potassium and calcium nitrates, found nitrogen fixation taking place. He measured it by the increase in the total nitrogen present at the close of the experiment as compared with that at the start. Table 1 is taken from his data and shows fixation when a large nitrogen ration is supplied.

✓Woods (50) (of Connecticut) grew scarlet clover in sand with a nutritive solution and accounted for all the nitrogen in the experiment, as did Atwater, by analysis of material at the outset and at the close. With no nitrogen in the solution, 18 plants fixed an average of 37 mgm. per plant. When 40 mgm. of nitrogen as calcium nitrate and potassium nitrate were added at the start, the fixation was reduced to 30 mgm. per plant. Vetch responded differently, fixing 20 mgm. in the former case and 47 in the latter. Cowpeas treated with nitrate fixed amounts varying from 87 to 129 mgm. of nitrogen. His data show a larger part of the nitrogen in the roots when nitrate was omitted than when it was added. He says, "all the plants grown without added nitrogen gained in nitrogen. Some of the plants supplied with nitrate showed a loss. The gain has occurred where root nodules are developed and without them there was no gain of any account."

Prazmowski (42) worked on an experiment similar to that of Woods to find out if the nitrates in the soil hindered or aided the bacteria in entering the plant and fixing nitrogen. Peas in sterile sand cultures containing 300 mgm. of nitrogen fixed 50 mgm. With no nitrogen in the sand and only 12 mgm. in the seed, 70 mgm. were taken from the air. In working with water cultures, nitrates held down nitrogen fixation and nodule production. In the absence of nitrates the fixation varied from 17 to 83 mgm. per plant. He says that the nitrogen content of the soil influences the time at which the nodule empties itself. The presence of nitrogen in the soil brings this change at the time of seed formation, but its absence from the soil permits the process of nodule growth and nitrogen fixation to go on slowly during the life of the plant with an increase in it at the time of seed formation.

TABLE 1

Nitrogen fixed by peas grown in nutrient solutions containing nitrates
(Taken from Atwater)

	NUMBER OF EXPERIMENT	NITROGEN SUPPLIED			NITROGEN AT CLOSE			GAIN OR LOSS OF NITROGEN
		In seeds	In solution	Total	Vines, roots, etc.	Residual solution	Total	
		mgm	*mgm.*	*mgm*	*mgm*	*mgm.*	*mgm.*	*mgm.*
Group I. Small nitrogen ration	1	36.7	59.4	96.1	116.4	1.4	117.8	−21.7
	3	72.6	59.4	132.0	158.9	3.8	162.7	−30.7
	5	34.2	59.4	93.6	156.1	0.0	156.1	−62.5
	7	71.5	59.4	130.9	158.1	0.0	158.1	−27.2
	9	35.3	59.4	94.7	186.5	1.4	187.9	−93.2
	11	72.5	59.4	131.9	210.9	2.7	213.6	−81.7
Group II Large nitrogen ration	2	34.4	136.9	171.3	178.9	2.0	180.9	−9.6
	4	75.2	136.9	212.1	200.6	12.8	213.4	−1.3
	6	34.8	136.9	171.7	149.6	1.2	150.8	−20.9
	8	70.3	136.9	207.2	197.5	12.7	210.2	−3.0
	10	34.6	136.9	171.5	277.8	35.7	313.5	−142.0
	12	68.8	136.9	205.7	260.2	45.7	305.9	−100.2

This work cited last, gives a different degree of effect for nitrates in a sand or open medium than it does for nitrates in water cultures. This fact may be of significance in explaining the injurious effects on nodule growth and nitrogen fixation which are attributed in many cases to the nitrates. The use of solutions may be inadequate for an experiment of this nature.

To test the effects of different forms of nitrogen on the nodule growth of legumes, Frank (14) used calcium nitrate, ammonium sulfate and urea. He analyzed seeds at the start and total plants at the close, and found that for the lupine the greatest growth and nitrogen increase in the plant, as well as the most profuse nodule production, took place in the absence of all nitrogenous compounds. The pea behaved similarly. He measured the nitrogen fixed in sand and soil, by lupines, peas and red clover, obtaining the results given in table 2.

Frank's data show that the yellow lupine fixes less nitrogen on a humus soil than on a sand soil, or that soils with a higher nitrogen content fix less nitrogen with this legume. It may be possible that physical differences or other factors were responsible, for he gives no detailed description of relations other than nitrogen in the soil.

For the peas and clover the case is different. Both gave a decided increase in the soil rich in nitrogen. For the lupine he believes that its nitrogen-fixing power is less in a nitrogen-rich soil than in one very poor in this respect. Nevertheless, a soil already rich in nitrogen may be enriched in this element by means of legumes. Peas and clover, he believes, reach their maximum fixing capacity only when using nitrogenous substances, especially nitrates, to supplement the bacteria on their roots, even though the direct opposite is true for the yellow lupine. The nitrogen enriching effect of legumes takes place not only in soils poor in nitrogen, but also in the better soils, rich in humus.

TABLE 2

Nitrogen fixed in sand and soil by lupines, peas and red clover
(Taken from Frank)

KIND OF SOIL	NUMBER OF PLANTS	DRY WEIGHT HARVESTED	NITROGEN		INCREASE IN FOLD	NITROGEN IN SOIL		INFECTION
			In seed and inoculum	In harvest		Outset	Close	
Lupine								
		gm	*gm.*	*gm*		*per cent*	*per cent*	
Sand	5	14 76	0 035	0 3609	10 3	0 0096	0 0157	1 large nodule
Humus soil . .	4	23 32	0 0364	0 2816	7 7	0 1076	0 1208	1 to 7 nodules
Peas								
Humus soil . . .	3	37 98	0 0282	0 7467	26 5	0 1076	0 1253	Of bean size
Red clover								
Sand .		44 33	0 0457	0 7087	15 5	0 0073	0 0105	Rich in nodules
Sterilized sand not inoculated... .		7 18	0 0457	0 0687	1 5	0 0073	0 0079	Few nodules
Humus soil		222 02	0 0457	4 6406	105 5	0 1076	0 1184	Few nodules

The above conclusion is quite the opposite to that of Maercker (31), who used the yellow lupine in sterilized and inoculated sand with varying amounts of potassium nitrate added. He found in this experiment that nitrates did not hinder or lessen the ability of the lupine as a nitrogen fixer. Nobbe and Hiltner (35) reported the diameter of *Robinia* nodules as 8 mm. in nitrogen-free soil, and 0.5 mm. in soil treated with nitrates.

Perhaps the most careful work in the early study of nitrogen fixation is that of Aeby (1) in his attempt to see if non-legumes would give nitrogen fixation. He used two soils, one a clay soil with 0.0783 per cent nitrogen and the other a "humus-rich" soil with 0.4050 per cent nitrogen. They were used in growing peas without nitrogen treatment, and with nitrogen added at four intervals to make a total of 2 gm. per pot of 4 kgm. of soil. Analyses were made for total nitrogen in all materials at the beginning and at the close. Any increase present at the close over that at the beginning represented fixation of nitrogen. This method of analysis gave a loss of nitrogen in fallow pots but a decided increase for those on which peas were grown. Peas growing in a rich soil fixed 1.976 gm. nitrogen per pot, while in the same soil treated with nitrogen, the fixation was but 1.621 gm. In the clay soil the corresponding figures are 2.759 gm. and 1.987 gm., respectively; a decrease of 0.355 and 0.772 gm. due to the nitrogen added. This indicates that in the soil rich, as well as one poor in nitrogen the addition of nitrogen depressed the fixation of atmospheric nitrogen. Furthermore, in the soil which was low in nitrogen the amount taken from the air by the peas was greater than in the rich soil, both when untreated and treated with nitrogen. Accordingly there was less fixation with increased amounts of nitrogen in the soils, which agrees with some of the preceding works cited. In the soil left fallow, he failed to recover as much nitrogen at the close as was present at the beginning. In the distribution of nitrogen in the roots and tops of plants, his results agree with those of Woods.

Nobbe and Hiltner (36) go farther in their statements than many others and conclude from a study of cross inoculation, that nodules have no influence on plant growth when plenty of soil nitrogen is available.

Salts containing nitrogen were used by Marchall (32) and found to inhibit nodule production in the following concentrations: alkaline nitrates 1 part in 10,000 and ammonium salts 1 part in 2000. By using soybeans and measuring the nitrogen increase in terms of the crop, when sterile or inoculated, or treated with nitrate nitrogen, Nobbe and Richter (38) found that with increased amounts of soluble nitrogen or humus substance added, the total nitrogen content of the crop decreased. They believed that small amounts of soluble nitrogen are beneficial to the young plants—at least until bacteroids are formed. According to them, inoculation was best in the absence of nitrates and decreased with the increase of the latter.

To judge the value of legumes as nitrogen fixers, Wohltmann and Bergene (51) used a variety of soils ranging in nitrogen from 0.046 per cent to 0.205 per cent and a peat soil with 1.650 per cent. These soils were treated with either ammonium nitrate or ammonium sulfate and planted to a number of legumes. They judged the amount of nitrogen taken from the air in terms of the number of nodules produced, and give the following results and conclusions in regard to the influence of the nitrogen in the soil on the amount of nitrogen fixed. On all soils to which ammonium nitrate was added the nodules failed to develop and the plants grew well in the absence of them. Ammonium sulfate suppressed nodule production completely in nine cases and almost completely in two cases.

These men believe that legumes do not need the help of bacteria and atmospheric nitrogen when there is present in the soil an abundant supply of available nitrogen, and that they use soil nitrogen almost exclusively. According to these conditions, legumes used as green manure would not add nitrogen to that soil whose available supply of this essential element is high. They point out that it is the "available" nitrogen rather than the "total" nitrogen that has a detrimental influence on nitrogen fixation.

Similar results were obtained about 1904 by Nobbe and Richter (39) who undertook to determine the effect of soluble nitrogen in soil on the amount of nitrogen taken from the air by vetch. They measured fixation by difference between total nitrogen in plants that were inoculated and those that were not so treated. The increase in the nitrogen content caused by bacteria was considered as coming from the air. Increasing amounts of potassium nitrate were added to the soil and the entire plants analyzed. They found the results given in table 3.

TABLE 3

Nitrogen in vetch, inoculated and uninoculated, treated with nitrate nitrogen

(From Nobbe and Richter)

	NO NITROGEN ADDED	500 MGM NITROGEN ADDED	1000 MGM. NITROGEN ADDED
Inoculated (gm.)	1 533	1 887	2 295
Not inoculated (gm)	0 095	0 389	0 618
Difference (gm)	1 438	1 498	1 677
Increase (per cent of total)	93 8	79 38	73 07
Nitrogen ratio	1.16 1	1:4 8	1:3 7

According to their figures the effect of inoculation decreases with the increase in soluble soil nitrogen. Where no soluble nitrogen was added the bacteria increased the nitrogen in the plant sixteen-fold. As much as 93 per cent of the nitrogen in the inoculated legume came from the atmosphere. With the addition of 500 mgm. of soluble nitrogen the increase by bacteria was almost four-fold, or about 80 per cent of the nitrogen in the entire plant came from the air. When more nitrate was added, the depressive effect increased, though not proportionally. These amounts of soluble nitrogen, according to their data, decreased the action of the bacteria in supplying the plant with atmospheric nitrogen.

A large variety of nitrogenous compounds were tested by Flamand (12) for their influence on nodule production with peas, beans and vetch. He found that potassium nitrate prohibited nodule formation when used in amounts as low as 1 part in 10,000; sodium nitrate required but 1 part in

2000, or the equivalent of 1000 pounds per acre. Urea, oxamide, and potassium cyanide were very prejudicial to the production of nodules even when used in very dilute solution. Ammonium salts, either as nitrate or sulfate, prohibited nodules on vetch. Table 4 gives the proportions in which these compounds were fatal to nodule production.

This work was done in water cultures and as in previous works cited may have given more significant effects than would be true of soils.

The contention that assimilable nitrogen hinders nodule production is further supported by A. Hercke (20) who concludes that, "when the soil contains sufficient assimilable nitrogen, the presence of nodules on the roots has no influence on the nitrogen content of lupines. When soil is poor in nitrogen the presence of nodules increases the absolute as well as the percentage of nitrogen content of the plant." This is contradicted by S. Hercke (21) who found that nitrogen compounds as ammonium sulfate, potassium nitrate and asparagin favored the growth of nodule bacteria.

TABLE 4

Nitrogen concentrations fatal to nodule production

(From Flamand)

	Pisum sativum	*Vicia narbonensis*	*Faba equina*
Potassium nitrate	1:10,000	1:10,000	1:10,000
Sodium nitrate	1:10,000	1:2,000	1:2,000
Calcium nitrate	1:2,000	1:10,000	1:20,000
Ammonium nitrate	1:10,000	1:20,000	1:2,000
Ammonium sulfate	1:10,000	1:20,000	1:10,000

Fred and Graul (17) tested the effect of soluble nitrogenous compounds on nitrogen fixation in different kinds of soil and with different kinds of plants. In general, they found that increasing amounts of soluble nitrogen depressed nodule production, but for this effect a larger amount of soluble nitrogen was necessary than would probably ever occur under field conditions. They are supported in this by the field experiments of Ewart (11).

When it was found that bacteria are responsible for nitrogen fixation in conjunction with the plant, the question arose concerning their behavior in this respect independent of a host. Several researches have been carried out to see how nitrogen in various forms in the media influences the nitrogen-assimilating capacity of this organism when separated from the plant. In 1891 Beijerinck (4) found nitrogen fixation taking place by these bacteria (since named *Ps. radicicola*) independent of a plant, when the media contained ammonium, sodium, and potassium nitrates. Other early workers who reported fixation independent of the plant in the presence of nitrogenous compounds were Prazmowski (42), Berthelot (5), and Frank (14), but they said it was too small to be significant. Larger nitrogen fixation was reported for the organism living independently by Maze (33) and by Lewis and Nicholson (29), the former finding as much as 23 mgm. of nitrogen fixed in 100 cc. of medium in 16 days.

The presence of nitrogen is considered injurious to the bacteria themselves according to Moore (34). He believes that, "the cultivation of bacteria upon media containing appreciable quantities of nitrogen for any length of time is sufficient to cause them to lose both the power of infection and that of fixing atmospheric nitrogen." This is strongly refuted, however, by Burrill and Hansen (7) who grew *Ps. radicicola* on nitrogen media for 30 months without a loss of its special adaptations or its capacity for producing nodules.

Very recently Fred (15) found that *Ps. radicicola* fixed more nitrogen when a trace of this element was present in the medium, but larger amounts of it retarded the process. He is supported by still more recent work of Hills (23) who found that fixation independent of the plant was increased by nitrate nitrogen. His results vary somewhat, and the fixation is large enough to be significant, which may not be wholly true of Fred's results.

This review of literature indicates that on the fixation of nitrogen by legumes, one may expect a significant influence from the nitrogen content of the soil. According to several researches soluble or assimilable nitrogen has a depressing influence, especially in water cultures and in soils when used in larger amounts. Nobbe and Hiltner (37) showed that the water-culture method itself was unfavorable for the development of nodules, and consequently nitrogen additions under such conditions may have been wrongly interpreted. The use of soil seems to be the best procedure and duplicates most nearly the field relations. Little has been done, however, in using carefully analyzed soil to measure the influence of soil nitrogen on the process of nitrogen fixation under those conditions generally prevailing in the soil. The opinions on this question are varied, though the more recent ones emphasize the assimilable or soluble nitrogen of the soil as being depressive to the efficiency of nitrogen-fixing bacteria. In most experiments cited the soluble nitrogen was applied as ammonia or nitrate salts and no account was taken of the nitrogen in organic matter. Such works fail to settle the significance of the organic matter in the soil concerning symbiotic nitrogen fixation.

III. EXPERIMENTAL

The following experimental work was undertaken in order to study further the relation of symbiotic nitrogen fixation to the nitrogen content of soils, especially the total nitrogen as obtained by usual soil analysis. In advising the use of legumes in a rotation cropping system, especially for the soils of the Corn Belt, which are moderately high in total nitrogen, the following questions often arise: Will the legumes fix any atmospheric nitrogen in a soil already containing large amounts of this element? If they do, how efficient will they be, and what significance does the nitrogen content of the soil have on the process? The following experimental work was undertaken with the hope of contributing a possible answer to these questions.

Plan of the experiment

In experimental work necessitating the measurement of nitrogen fixation, two general methods have been widely accepted. The first one consists in growing one set of legume plants without bacteria on the roots, and another set under like conditions but with the organism applied. The difference in the nitrogen content of the two sets of plants represents the increase due to bacteria The second method consists of careful analyses of all materials to determine the total nitrogen present both at the outset and at the close of the experiment. Under carefully controlled conditions, with no nitrogen added, the increase of this element at the close over that at the beginning represents nitrogen drawn from the atmosphere.

Objections have been made to the first method on the ground that the soil or medium in which the plant grows is not taken into consideration. An increase in the nitrogen content of the plant because of the bacteria may mean that the plant was able to take more nitrogen from the soil and does not prove that all such increase in nitrogen came from the air.

The second method is more detailed and painstaking and gives difficulty because of lack of refined methods for determining total nitrogen. However, it measures the total nitrogen in all materials concerned at the beginning, and again at the close, so that the increase must come from some other source than the soil. In carefully controlled conditions this source must be the atmosphere. The latter method was used in this study.

The soil used for the pot cultures was a yellow silt loam from the unglaciated area of southern Illinois and contained 625 pounds of nitrogen per 2,000,000 pounds of surface soil. It was extremely low in organic matter, hence in such a poor physical condition that at first sight it would appear much like a clay or clay loam. It gave an acid reaction, and in order to neutralize this it was treated with calcium carbonate at the rate of 2 tons per acre. Plant nutrient elements other than nitrogen were added in soluble form during the time the plants were growing to assure good fertility, except for nitrogen which is the main deficiency in the soil (26). This soil was chosen because it was so low in nitrogen, and would offer a low nitrogen basis, which had become stable because of years of weathering, and which could be increased by several large increments without surpassing the nitrogen content of the more fertile soils.

Two kinds of legumes were grown, soybeans and cowpeas. The first series included soybeans but failed to do as well as expected in greenhouse work. They were replaced by cowpeas for the later series which were grown out-of-doors during most of the time. Insects molested the plants in all series and were combatted with chemicals containing no nitrogen. Red spiders were especially prevalent and were sprayed with cold water or potassium sulfide solution. During the first series a fungus gnat (*Sciaria mycetophilidae*) (18) infested the soils which were high in organic matter. Later troubles from this insect were not experienced. Insect infestations were prevalent on all plants, so that even though they may have hindered growth to some extent, their disturbances would not prevent comparable results.

In the first series which contained soybeans, four duplicate series of 1-gallon pots were used with the soil treatments given in table 5.

TABLE 5

Soil treatments in crop series 1
(Soybeans)

POT	NITROGEN ADDED TO SOIL (POUNDS PER 2,000,000 POUNDS OF SOIL)
	Pot series 1
1-2	None (not inoculated)
3-4	None
5-6	10 pounds as nitrate
7-8	50 pounds as nitrate
9-10	150 pounds as nitrate
	Pot series 2
11-12	1000 pounds as clover tops
13-14	2000 pounds as clover tops
15-16	3000 pounds as clover tops
17-18	4000 pounds as clover tops
19-20	5000 pounds as clover tops
	Pot series 3
21-22	1000 pounds as clover tops and 10 pounds as nitrate
23-24	2000 pounds as clover tops and 10 pounds as nitrate
25-26	3000 pounds as clover tops and 10 pounds as nitrate
27-28	4000 pounds as clover tops and 10 pounds as nitrate
29-30	5000 pounds as clover tops and 10 pounds as nitrate
	Pot series 4
31-32	1000 pounds as clover tops and 50 pounds as nitrate
33-34	2000 pounds as clover tops and 50 pounds as nitrate
35-36	3000 pounds as clover tops and 50 pounds as nitrate
37-38	4000 pounds as clover tops and 50 pounds as nitrate
39-40	5000 pounds as clover tops and 50 pounds as nitrate

The addition of nitrate nitrogen and clover tops was made on the basis of one acre, or 2,000,000 pounds of soil. In all cases a constant amount of soil was used, and the increasing amounts of clover tops added gave increasing amounts of substrate for the plants in the series. This treatment also brought about decided changes in the soil's physical condition. Four pots were used as checks. All pots in the series were inoculated except two of the check pots, no. 3 and 4, which remained uncontaminated and showed no nodules at the close of the experiment.

TABLE 6

Soil treatments in crop series 2
(Cowpeas)

POT	NITROGEN ADDED TO SOIL (POUNDS PER 2,000,000 POUNDS OF SOIL)
	Pot series 1
1a–2a	None
3a–4a	10 pounds as nitrate
5a–6a	25 pounds as nitrate
7a–8a	50 pounds as nitrate
9a–10a	100 pounds as nitrate
	Pot series 2
1–2	None (not inoculated)
3–4	None
11–12	1000 pounds as clover tops
13–14	2000 pounds as clover tops
15–16	3000 pounds as clover tops
17 18	4000 pounds as clover tops
19 20	5000 pounds as clover tops

TABLE 7

Soil treatments in crop series 3
(Cowpeas)

POT	NITROGEN ADDED TO SOIL (POUNDS PER 2,000,000 POUNDS OF SOIL)
	Pot series 1
1b–2b	None
3b–4b	50 pounds as nitrates
5b–6b	100 pounds as nitrates
7b–8b	150 pounds as nitrates
9b–10b	200 pounds as nitrates
11b–12b	250 pounds as nitrates
13b–14b	50 pounds as nitrates (added at intervals)
	Pot series 2
1–2	None
3–4	None
11–12	1000 pounds as clover tops
13–14	2000 pounds as clover tops
15–16	3000 pounds as clover tops
17–18	4000 pounds as clover tops
19–20	5000 pounds as clover tops

The second series was seeded with cowpeas, and contained two pot series, one treated with nitrates, and one with organic matter. Original soil, the same as used previously, was treated with nitrate nitrogen, while the series treated with organic matter consisted of a part of those soils used in the first crop series The four check pots and the ten pots that had been treated with nitrogen as clover tops only, were taken to complete this series. These soils were in better physical condition and the stage of most rapid decay seemed to have been passed. Constant amounts of soil (3300 gm. water-free soil) were weighed into each pot. All pots were inoculated except the two check pots, but evidently these were already inoculated, or became contaminated, for nodules were present at the close of the series. This crop series with its treatment is summarized in table 6.

The third series was similar in soil treatment to that of the second. Some of the original soil was treated with nitrates for one pot series, while those soils which had been used in both previous series were used for the other pot series. Cowpeas were grown as the crop. The treatments are given in table 7.

The last two series in which cowpeas were grown were kept out-of-doors near the greenhouse for much of the time. This seemed to favor better growth, and lessened the insect troubles. Care was taken to move the plants inside before rain and only once did rain fall on each series. It was only a slight shower and the effects in adding nitrogen were negligible.

Analytical methods

The usual analytical methods were employed. To measure the total nitrogen in organic matter such as seeds and plants, the materials were digested with mercury and sulfuric acid or with acid and sodium sulfate, neutralized with sodium hydroxide and potassium sulfide, distilled into standard acid and titrated with standard alkali. For the total nitrogen determination on soils, 10-gm. samples were first dried for 8 hours at a temperature of 107°-108°C., the loss in moisture determined, and then transferred for digestion. Sulfuric acid containing salicylic acid was added and allowed to stand for some time, usually over night. Sodium thiosulfate was introduced and heated slowly until frothing ceased. Mercury was then added and digested to clearness, potassium permanganate being used to assure complete oxidation. This was then neutralized with alkali and distilled into standard acid, according to the usual procedure.

For the determination of nitrate nitrogen, samples of soil were dried at 107°-108°C. for 8 hours and then extracted with 0.0625 *N* hydrochloric acid. After being made alkaline, the ammonia was boiled off, and the original volume replaced by nitrogen-free water. Devarda's metal was added, the reduced nitrogen distilled into 0.0357 *N* standard sulfuric acid and titrated.

In all the determinations the greatest care was exercised. The organic matter was dried, weighed, thoroughly ground, and kept in air-tight containers to prevent decided variations in moisture content before an entire series could be analyzed. Purest chemicals were used and all reagents were made up in large amounts to insure greater uniformity in results. Standard acid for nitrogen in soil was of 1/14 normality and with sodium alizarin sulfonate as an indicator, titrations checked within 0.2 cc., a variation equivalent to 0.2 mgm. nitrogen in 10-gm. samples, or 40 pounds of nitrogen in 2,000,000 pounds of soil. For nitrate determinations, a sulfuric acid of 1/28 normality was used and 0.2 cc. allowed as variation in duplicates. Determinations in moisture loss for 10-gm. samples were usually less than 6 mgm. All calculations and determinations for soil were on the water-free basis to insure uniformity. Samples were run in triplicate and quadruplicate to offset errors. Only doubly distilled nitrogen-free water was used, both in analytical work and in the pot cultures.

Discussion of crop series

Series 1 (Soybeans)

The soil for this series was thoroughly mixed in a quantity large enough for all the pots. To determine the nitrogen in it, a large sample was taken and ground to pass a 100-mesh sieve and then analyzed for total nitrogen. According to the analysis, 30-gm. quantities of air-dry soil, which were equivalent to 29.1970 gm. water-free soil, contained 9.1568 mgm., or 0.03136 per cent nitrogen on the latter basis, as an average of five determinations varying less than 0.2 mgm. Each pot received 3500 gm. of soil with a moisture content of 14.01 per cent, or the equivalent of 3421 gm. of water-free soil, and a nit ogen content according to the above analysis of 1072 mgm.

For one pot series the soil was treated with nitrate nitrogen only, at the rate of 17.2, 86.0 and 258 mgm of nitrogen per pot, or the equivalent of 10, 50 and 150 pounds per 2,000,000 pounds of soil. For three additional pot series the nitrogen in the soil was increased at increments equivalent to 1,000 pounds of nitrogen per 2,000,000 pounds of soil by means of finely ground clover tops. The clover tops contained 2.709 per cent of nitrogen, as an average of 12 analyses. With 3500 gm. of soil per pot an increment of 1000 pounds per 2,000,000—one part in 2000—required 1.75 gm. of nitrogen, or 64.6 gm. of clover tops. Accordingly, for the second pot series enough clover tops were added to give a series of the original soil, whose nitrogen was increased at the rate of 1000, 2000, 3000, 4000 and 5000 pounds per acre. The third series was similar to the second except that in addition to the organic matter sodium nitrate also was added to all the pots at the rate of 10 pounds of nitrogen per 2,000,000 pounds of soil, while the fourth series differed from the third only by the addition of sodium nitrate at the rate of 50 pounds of nitrogen instead of 10. This gave one pot series whose nitrogen was increased as nitrate, one with increasing nitrogen in organic matter as clover tops, another increasing with clover tops and 10 pounds of nitrate nitrogen and the fourth whose nitrogen increased as clover tops and 50 pounds of nitrate nitrogen. The addition of varying amounts of clover tops to 3500 gm. of soil increased the amount of soil in the pots, so that the weight of soil in the different pots was not a constant figure.

According to Hilgard (22) and others, the best moisture content of soils for proper plant development is one-half of the moisture-holding capacity. On this basis, determinations of moisture-holding capacity were made on these soil series as modified by additions of clover tops, and the water applied to the pots in quantities to give the equivalent of 50 per cent of the moisture-holding capacity. An attempt was made to maintain such conditions throughout the experiment by weighing the pots at intervals, but the labor involved was large and the growth of the crops prohibited absolute accuracy. It was therefore necessary to apply the water according to one's best judgment. The presence of large amounts of organic matter caused trouble in keeping the moisture at optimum amounts.

Soybean seeds were selected so that each seed weighed 140 mgm. Six of these were planted in each pot, in order that five vigorous plants might be assured in spite of low vitality and poor germination. The sixth plant or seed was removed after sufficient time had elapsed for the plants to get well started. According to the analyses of the beans, each seed contained an average of 9 mgm. of nitrogen.

The growth of this series was fair. Numerous replantings were necessary because the large amounts of fresh organic matter interfered with proper germination of the seeds, a difficulty experienced also by Fred (16) at Wisconsin and by others at Illinois with oats, cowpeas and clover after turning under alfalfa. The injurious effects were more marked with the increase in organic matter so that on the pots receiving clover tops at the rate of 5000 pounds of nitrogen per 2,000,000 pounds of soil, only small plants were produced. These effects were not offset by the nitrate nitrogen added at the rate of 10 pounds and 50 pounds per 2,000,000 along with the organic matter. Some plants "damped-off" at the age of four weeks, while others died later. The growth was much influenced by the organic matter in the soil, being the best of the entire series where 1000 and 2000 pounds of nitrogen had been added as clover tops, but decidedly poorer in soil with larger amounts of this material added. The plants showed a less deep color, smaller leaves, less branching and more translocation, with the increase in organic matter above the equivalent of 2000 pounds of nitrogen per acre.

The effect of the nitrates was marked in the soil when these were added alone, but gave no appreciable effect when coupled with the organic matter. In the soil alone, the increasing amounts of nitrates gave improved plant growth for the early part of the plant season, but these differences were obliterated when the crop matured, as was shown by the weights and nitrogen content of the crop. The differences in the four pot series just before their close are shown in plate 1.

Nitrates in soil growing soybeans. Before removing the plants at harvest, samples of soil were taken and nitrates determined by extraction and reduction with Devarda's alloy. Forty-gram samples of water-free soil were used. Determinations on those pots with organic matter added were somewhat erratic and failed to check well in duplicate. Since large amounts of organic matter are known to interfere with reduction methods (9, 48) and give high results, full credence cannot be placed on the results from these soils with organic matter. Table 8 gives the results of the determinations of nitrates.

TABLE 8

Nitrate nitrogen in soil growing soybeans

POT	NITROGEN ADDED (POUNDS PER 2,000,000 POUNDS OF SOIL)	MILLIGRAMS IN 40 GRAM SOIL	MILLIGRAMS PER POT	POUNDS PER 2,000,000 POUNDS OF SOIL
1	None	0 337	28	16.8
2	None	0 366	31	18.3
3	None	0 378	32	18.9
4	None	0 337	28	16.8
5	10 pounds as nitrate	0 397	33	19.8
6	10 pounds as nitrate	0 378	32	18.9
7	50 pounds as nitrate	0 337	28	16.8
8	50 pounds as nitrate	0 397	33	19.8
9	150 pounds as nitrate	1.353	115	67.6
10	150 pounds as nitrate	1 533	131	76.6
11	1000 pounds as clover tops	2 031	176	100.0
12	1000 pounds as clover tops	0 816	71	40.0
13	2000 pounds as clover tops	2 442	216	122.0
14	2000 pounds as clover tops	5.835	516	291.0
15	3000 pounds as clover tops	15 852	1425	792.0
16	3000 pounds as clover tops	12 585	1132	629.0
17	4000 pounds as clover tops	9 459	864	472.0
18	4000 pounds as clover tops	9.757	892	487.0
19	5000 pounds as clover tops	8 403	780	420 0
20	5000 pounds as clover tops	8 722	810	436 0

From the above table it is evident that the pots receiving the equivalent of 10 and 50 pounds of nitrate nitrogen per acre contained no more nitrogen in this form at the close of the series than the pots left untreated. Evidently the plants consumed all that was applied, since soil conditions were unfavorable to denitrification, the other possible chance for nitrate removal. With the pots receiving the equivalent of 150 pounds per acre the conditions were different and the nitrate content was much higher. The application of such a large amount, far above that needed for the plant growth, left some in the soil, since no leaching took place and the open soil prohibited denitrification. This would suggest that such a large application supplies more nitrogen than a single crop of soybeans can remove.

In the soils with organic matter the figures indicate excessive amounts of nitrates, reaching the maximum in the pots receiving 3000 pounds of nitrogen as clover tops per 2,000,000 pounds of soil, and decreasing somewhat with the heavier applications. The high results may have been caused partly by the interference of the organic matter.

Nodule production by soybeans. When the plants were harvested, the roots were removed from the soil as completely as possible and studied for nodule production. Pots 1 and 2, untreated, which had not been inoculated at the outset were still sterile in respect to the soybean bacteria, containing no nodules and showing no contamination from the inoculated pots all about them. This indicates that there is no great danger of inoculation by contamination in an experiment of short duration of this kind with soybeans when no special precautions are taken. In the pots in this series, treated with sodium nitrate, many good-sized nodules were present giving no visible effect from the nitrate treatment either on the size or on the number of the nodules, except perhaps in the two pots receiving the equivalent of 150 pounds of nitrogen per acre. In these the nodules were smaller, but this difference was offset by increased numbers.

In the pot series treated with clover tops, nodule production decreased with added amounts of organic matter. However, in the soils with the highest application there was such a poor development of the plants that very few nodules could be expected. The low vitality and poor plant growth rather than any disturbing factors in the soil may have been the cause of insignificant nodule formation. There was no evidence to indicate that with healthy plants nodule production would have been prohibited by these large amounts of organic matter.

In the soil treated with such large amounts of clover tops as would give 5000 pounds of nitrogen in 2,000,000 pounds of soil, the original composition and physical make-up were radically altered. Such modifications of soil condigions invited the infestation by a winged fungus gnat (*Sciara mycetophilidae*) which may have consumed and removed much of the nitrogen. The open soil structure and a plentiful food supply in the form of decaying organic matter furnished an excellent habitat for these small insects. The infestation originated in the pots with the heaviest applications of organic matter, but spread to all pots so treated. Its duration was limited to a certain stage or period in the decomposition of the organic matter, and the insects were present only long enough to develop the larvae and pupae of one generation. They may have been a disturbing factor of which no account could be taken, and may have been partly responsible for the losses of nitrogen in these series.

In tables 9, 10, 11 and 12 is given the nitrogen balance for the four pot series with soybeans. Many of the analytical data are omitted and only the summation figures are given to show the balance between the total nitrogen in the soil and seed at the beginning, and that of the soil and crop at the close. Figures given are the results of quadruplicate determinations in the soil, while in the crop the total material was analyzed. Four pots given in the first series are used as the checks for the remaining pot series.

Following the tables is a graphical representation of the increases and losses in total nitrogen shown in the tables. The ordinates represent milligrams of nitrogen gained or lost while the abscissas represent nitrate nitrogen added to the soil in case of one curve, and the total nitrogen present in the soil at the outset for the remaining curves.

Instead of showing an increase in nitrogen, most pots show a decrease. Increases in nitrogen, when they occur, are insignificant as compared with the losses. When the soil was treated with nitrates only, one of the duplicate pots showed an increase in nitrogen at the close over that present at the outset, but this increase was within the limit of difference between duplicate determinations which, when calculated per pot, was 60 mgm. of nitrogen. The limits of error in nitrogen per pot were less, however, than this figure, as a result of quadruplicate analysis. The largest loss of nitrogen was 127 mgm. As more nitrate nitrogen was added at the beginning, the loss at the close increased, corresponding closely to the amount added. This indicates that perhaps the

TABLE 9

Nitrogen balance—Soybeans

Pot series 1, soil treated with nitrates

POT	NITRATE NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	NITROGEN IN POT AT CLOSE	CROP WEIGHT	NITROGEN IN CROP	NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE OR FIXATION	AVERAGE
	mgm	*gm*	*mgm*	*mgm*	*gm.*	*mgm.*	*mgm*	*mgm.*	*mgm.*	*mgm.*
1	None (uninoculated)	9.8394[a]	2.8713[a]	998[b]	2.9	36[c]	1034	1117[d]	−83	−36
2	None (uninoculated)	9.8215	2.9714	1032	4.0	58	1090	1117	−55	
3	None	9.8260	2.9848	1040	3.5	98	1138	1117	21	
4	None	9.8788	2.8864	1003	3.85	89	1092	1117	−25	
5	17.2	9.7864	2.8783	1005	3.85	95	1100	1134	−34	−16
6	17.2	9.8025	2.9714	1037	4.75	98	1135	1134	− 1	
7	86	9.8029	2.9140	1016	5.12	113	1129	1203	−74	−93
8	86	9.8183	2.8498	992	4.25	98	1090	1203	−113	
9	258	9.8087	3.2243	1124	4.25	124	1248	1375	−127	−122
10	258	9.8190	3.1790	1106	3.70	151	1257	1375	−118	

[a] Average of four determinations, or duplicates made at two different times.

[b] Based on 3421 gm. water-free soil in each pot.

[c] Found by analyzing entire crop, roots and tops.

[d] Based on the analyses of the soil at the outset giving 0.03136 per cent of nitrogen, or 1072 mgm. of nitrogen in each pot with 3421 gm. of soil, and 5 seeds containing 45 mgm. of nitrogen. Additions of nitrate were equivalent to 17.2, 86 and 258 mgm. of nitrogen.

analytical procedure failed to detect the nitrate nitrogen added. Had the nitrate nitrogen been incorporated into the plant tissue as protein, it would certainly have been found; but it might be possible, in spite of all care in this respect, that such small amounts of nitrate were not detected by the analysis for total nitrogen at the close. Later crop series receiving far larger amounts of nitrogen as nitrate, failed to show any such discrepancies.

TABLE 10

Nitrogen balance—Soybeans

Pot series 2, soil treated with clovei tops

POT	NITROGEN ADDED AS CLOVER TOPS	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	SOIL IN POT	NITROGEN IN POT AT CLOSE	CROP WEIGHT	NITROGEN IN CROP	NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
	mgm	gm.	mgm.	gm	mgm.	gm.	mgm.	mgm	mgm.	mgm.	mgm.
11	1750[a]	9 8361[b]	7.7461[b]	3480	2740[c]	2 85	122	2862	2867	−5	79
12	1750	9 8385	8.0541	3480	2848	4.70	182	3030	2867	163	
13	3500	9 8279	12 3984	3539	4464	4 15	189	4653	4617	36	90
14	3500	9.8216	12 7212	3539	4583	4 05	179	4762	4617	145	
15	5250	9 7951	15 4721	3598	5683	3 55	199	5882	6367	−485	−351
16	5250	9 7905	16 3141	3598	5995	2.70	154	6149	6367	−218	
17	7000	9 7815	19.9884	3657	7473	1 85	131	7604	8117	−513	−673
18	7000	9 8248[f]	19 3026	3657	7184	1 70	99	7283	8117	834	
19	8750	9 7879	23 2323	3716	8820	1 25	91	8911	9867	−956	−951
20	8750	9 8146	23 3436	3716	8838	1.20	82	8920	9867	−947	

[a] Clover tops added were figured on the basis of 3500 gm. of soil per pot instead of 3421, hence are not truly equivalent to the number of pounds per 2,000,000 pounds of soil as the increments were intended.

[b] Average of four determinations, or duplicates made at two different times.

[c] Based on water-free soil in pot as given in fifth column.

[d] Found by analyzing entire crop, roots and tops.

[e] Based on the analyses of the soil at the outset giving 0.03136 per cent of nitrogen, or 1072 mgm. of nitrogen in each pot with 3421 gm. of soil, and 5 seeds containing 45 mgm. of nitrogen.

[f] Two determinations only.

In the three series receiving organic matter alone and organic matter in conjunction with 10 and 50 pounds of nitrogen as nitrate, there was an increase in nitrogen for all pots receiving clover tops equal to 1000 pounds of nitrogen per acre, and for two pots treated with organic matter equivalent to 2000 pounds of nitrogen. All other pots in these three series showed decided losses. In the pots with the equivalent of 5000 pounds of nitrogen added as clover tops this loss ran as high as 1150 mgm. of nitrogen in some few cases.

These large losses must have resulted from the very rapid decomposition of the fresh organic matter and doubtless the nitrogen of the clover tops escaped as gaseous ammonia. The excessive application encouraged rapid decomposition, and with 320 gm. of clover tops incorporated in 3421 gm. of soil, the proportion of soil may have been too small to absorb all the ammonia. The losses increased fairly regularly with larger applications of clover tops, though in no definite mathematical relation.

TABLE 11

Nitrogen balance—Soybeans

Pot series 3, soil treated with clover tops and 10 pounds of nitrate nitrogen

POT	NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	SOIL IN POT	NITROGEN IN POT AT CLOSE	CROP WEIGHT	NITROGEN IN CROP	NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
	mgm	*gm*	*mgm*	*gm.*	*mgm*	*gm.*	*mgm*	*mgm*	*mgm*	*mgm.*	*mgm.*
21	1750[a] as clover tops and 17 2 as nitrate	9 7732[b]	7 9007[b]	3480	2813[c]	3 85	144[d]	2957	2884[e]	73	125
22		9 7804	8 1348	3480	2894	4 1	167	3061	2884	177	
23	3500 as clover tops and 17 2 as nitrate	9 7684	11 8428	3539	4290	4 2	167	4457	4634	−177	−116
24		9 7537	12 1518	3539	4409	3 9	169	4578	4634	−56	
25	5250 as clover tops and 17 2 as nitrate	9 7672	14 8770	3598	5480	2 2	123	5603	6384	−781	−616
26		9 7629	15 7567	3598	5806	2 0	126	5932	6384	−452	
27	7000 as clover tops and 17 2 as nitrate	9 7648	18 6387	3657	6980	1 9	105	7085	8134	−949	−876
28		9 7642	19 2363	3657	7204	1 85	126	7330	8134	−804	
29	8750 as clover tops and 17 2 as nitrate	9 7626	22 6532	3716	8628	0 05	73	8701	9884	−1183	−1152
30		9 7556	22 4843	3716	8653	1 80	110	8763	9884	−1121	

[a] Clover tops and nitrate added were figured on the basis of 3500 gm. of soil per pot instead of 3421, hence are not truly equivalent to the number of pounds per 2,000,000 pounds of soil as the increments were intended.

[b] Average of four determinations, or duplicates made at two different times.

[c] Based on weights of soil given in fifth column.

[d] Found by analyzing entire crop, roots and tops

[e] Nitrogen in soil 1072 mgm., nitrogen in seed 45 mgm., nitrogen in 64.59 gm. clover tops equivalent to 1000 pounds per acre 1750 mgm., and nitrogen as nitrate 17.2 mgm.

From the data of this series, one cannot say with any great certainty that the soybeans so grown used atmospheric nitrogen. The treatment of nitrate apparently prevented an increase in nitrogen while the treatment of clover tops equal to 1000 pounds of nitrogen gave an increase of nitrogen. This must have come from the air in the form of nitrogen fixation. The fact that there is a loss of total nitrogen in the system does not deny the possibility of the plants fixing nitrogen, for the legume may have used atmospheric nitrogen at the same time this escape from the soil was taking place.

The crop growth on all these series was small, and the total nitrogen in the crop of any pot never exceeded 200 mgm. With such small plant growth no great fixation was possible for the fixation of atmospheric nitrogen consists in the utilization of this form of nitrogen by the plant for tissue building, and unless plant growth is significant, no marked use of atmospheric nitrogen can be expected. The conditions of this part of the experiment gave too narrow a margin between the possible nitrogen fixed and the limits of variation, and

TABLE 12

Nitrogen balance—Soybeans

Pot series 4, soil treated with clover tops and 50 pounds of nitrate nitrogen

POT	NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	SOIL IN POT	NITROGEN IN POT AT CLOSE	CROP WEIGHT	NITROGEN IN CROP	NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
	mgm.	gm	mgm	gm	mgm	gm	mgm	mgm	mgm	mgm.	mgm
31	1750[a] as clover tops and 86 as nitrate	9.7603[b]	8.4575[b]	3480	3015[c]	3.6	154[d]	3169	2953[e]	216	114
32		9.7656	7.9314	3480	2826	3.3	140	2966	2953	13	
33	3500 as clover tops and 86 as nitrate	9.7694	12.0295	3539	4357	3.25	144	4501	4703	−202	−187
34		9.7561	12.0698	3539	4378	3.05	152	4530	4703	−173	
35	5250 as clover tops and 86 as nitrate	9.7481	15.0377	3598	5550	1.5	93	5643	6463	−820	−490
36		9.7474	16.7461	3598	6181	1.9	122	6303	6463	−160	
37	7000 as clover tops and 86 as nitrate	9.7386	19.6224	3657	7368	1.5	101	7469	8203	−734	−950
38		9.7546	18.4956	3657	6934	1.65	103	7037	8203	−1166	
39	8750 as clover tops and 86 as nitrate	9.7601	23.3127	3716	8875	0.9	47	8922	9953	−1031	−1148
40		9.7570	22.6049	3716	8609	1.05	79	8688	9953	−1265	

[a] Clover tops and nitrate added were figured on the basis of 3500 gm. of soil per pot instead of 3421, hence are not truly equivalent to the number of pounds per 2,000,000 pounds of soil as the increments were intended.

[b] Average of four determinations or duplicates made at two different times.

[c] Based on weights of soil given in fifth column.

[d] Found by analyzing entire crop, roots and tops.

[e] Nitrogen in soil 1072 mgm., nitrogen in seed 45 mgm., nitrogen in 64.59 gm. clover tops equivalent to 1000 pounds per acre 1750 mgm., and nitrogen as nitrate 86 mgm.

even though increases were shown in some cases, they failed to give assurance of any significant fixation. Unless a reasonable amount of fixation was taking place, the influence of the amount of nitrogen in the soil upon it could not be measured.

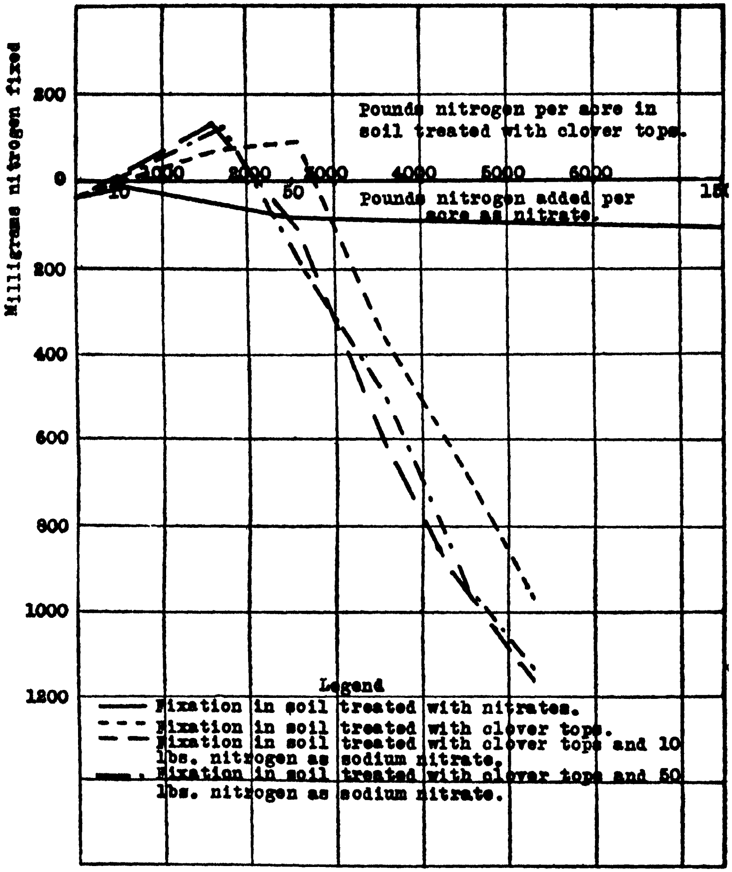

FIG. 1. GRAPHS SHOWING NITROGEN FIXATION BY SOYBEANS ON SOILS TREATED WITH NITRATES, CLOVER TOPS, AND WITH NITRATES AND CLOVER TOPS

Series 2 (Cowpeas)

For the second crop series cowpeas were grown in the hope that they would be less subject to the infestation by insects, more apt to do well under greenhouse conditions and more able as nitrogen fixers. Only two soil treatments were employed, one in which increasing amounts of nitrate nitrogen were added and the other one in which the nitrogen had been increased by organic matter.

Some of the original soil left over when the previous crop series was made up and stored in the dry condition was used for the treatment with nitrate nitrogen. The equivalent of 3300 gm. of water-free soil was weighed into each pot and enough sodium nitrate added in a solution to give increases in nitrogen equivalent to 10, 25, 50, and 100 pounds of nitrogen in 2,000,000 pounds of soil.

For the series whose nitrogen increase was in the form of organic matter, the soils used in the previous series in the pots 1 to 4 and 11 to 20, inclusive, were used again. Pots 1 to 4 had received no treatment with nitrogen and again served as checks. Pots 11 to 20 were also used without modification, save that the amount of soil in each pot was limited to 3300 gm. on the water-free basis. The nitrogen content in these soils no longer showed increments of 1000 pounds per 2,000,000 pounds of soil as a result of losses while growing soybeans, but were used because their differences in nitrogen content were very marked and they were similar in all respects except this one.

Plant-foods other than nitrogen were supplied in a soluble form. Calcium carbonate was added at the rate of 2 tons per 2,000,000 pounds.

Six cowpeas of uniform weight and known nitrogen content were planted in each pot and later when the plants were well started were reduced to five. The nitrogen added by the five cowpea seeds was equivalent to 43 mgm. During the early spring the pots were kept in the greenhouse but later were kept outdoors in a screened area. No difficulty in germination was experienced, and the growth was decidedly better than that of the soybeans.

Differences in these two series were soon noticeable. For the series receiving nitrate the plants were taller, the leaves larger and the color deeper where greater amounts of nitrate were added. These differences later disappeared so that by the time of harvest, there were no significant variations within the entire series, either in crop weight or total nitrogen.

The soil series treated with nothing but organic matter and which had been previously used for soybeans, gave a decidedly better growth of cowpeas than the series treated with nitrates. The differences are shown in plates 2 and 3. In this series the early growth was best in the pot receiving 1000 pounds of nitrogen per acre as clover tops. There was a decrease in growth with the increase of added organic matter, but even all these were equal to the check, receiving no organic matter. In the latter part of the growing season these differences were reversed, and the largest crop yields and the largest total amounts of nitrogen in the crop were produced on the pots receiving most organic matter. The crops were grown for about 135 days and then harvested. Some plants had produced blossoms and a few had set pods. The fact that the pots were much shaded by some large trees lengthened the vegetative period and delayed seed development.

When the crops were harvested the roots were removed as completely as possible and carefully examined for nodules. It is important to note that the nitrate series produced many nodules, even with 100 pounds of nitrogen in this form. Where organic matter was added, the nodules were most numerous in the lesser applications but were larger in size as the applications increased. There were no indications in any pots that insufficient nodules were present for nitrogen fixation. If the presence of the nodule is certain evidence that atmospheric nitrogen is used by the plant, then both these soil series permitted fixation to take place in all pots regardless of treatments.

Determinations of nitrate nitrogen in the soils in all the pots were made just before the plants were harvested. In the soil treated with sodium nitrate, no nitrogen in this form was found. The determinations of nitrates gave results no larger than those of the blank determinations. For the series with the organic matter, this treatment interfered with the analytical procedure for nitrates so that no definite statement of the amounts of nitrate present can be made Results were erratic, but indications pointed to the presence of comparatively large amounts of nitrate nitrogen. Evidently nitrification was going on and nitrates were present in the soil but this had not prohibited the production of many large-sized nodules on the roots of the cowpeas.

TABLE 13

Nitrogen balance—Cowpeas I

Pot series 1, soil treated with nitrates

POT	NITRATE NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	NITROGEN IN POT AT CLOSE	WEIGHT OF CROP		NITROGEN IN THE CROP		NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
					Tops	Roots	Tops	Roots				
	mgm.	gm	mgm	mgm	gm	gm	mgm.	mgm.	mgm	mgm	mgm	mgm.
1a	None	9 7673[a]	3 2755[a]	1106[b]	15 75	8 855	415	152	1673	1077[c]	596	688
2a	None	9 6763	3 7410	1275	14 75	9 435	418	164	1857	1077	780	
3a	16 5	9 7201	3 3414	1134	14 80	7 820	306	139	1579	1093	486	533
4a	16 5	9 7034	3 1594	1074	16 40	7 800	440	159	1673	1093	580	
5a	41 0	9 6933	3 1163	1061	14 45	7 145	292	147	1500	1118	382	512
6a	41 0	9 7568	3 3418	1130	17 65	7 781	445	185	1760	1118	642	
7a	82 0	9 7691	2 9373	992	16.15	8 408	404	162	1558	1159	399	453
8a	82 0	9 7215	3 1031	1053	16 65	7 675	445	168	1666	1159	507	
9a	157 0	9 7441	3 0102	1019	15 45	6 798	347	124	1490	1234	256	264
10a	157 0	9 7641	2 9108	983	14 80	5 610	385	138	1506	1234	272	

[a] Average of three determinations

[b] Calculated on the basis of 3300 gm. of water-free soil.

[c] Based on 3300 gm of water-free soil with 0 03136 per cent of nitrogen, or 1077 mgm. of nitrogen, and five cowpea seeds with 43 mgm. of nitrogen.

The harvested crops were thoroughly dried, weighed and later ground to permit uniform sampling. The crop growth was too large to be analyzed in total and only triplicate 2-gm. samples were used. The soil was air-dried, ground and sampled. Analyses were made by the same methods used in the previous series, but instead of using four samples, only triplicate determinations were made. On the basis of these analyses, the total nitrogen in the soil and the crop at the close of the series was calculated.

Tables 13 and 14 give the nitrogen balance for the second crop series with cowpeas. Only the summations of analytical data are given. Since the soils in pots 1 to 4 and 11 to 20 were analyzed at the close of the soybean series no analyses were made on these soils at the beginning of this series, but the amount of nitrogen in 3300 gm. of soil was calculated from the preceding analyses. The soil which had been stored and used in this series was analyzed and found to contain the same amount of nitrogen as at the beginning of the soybean series, or 0.03136 per cent of nitrogen on the water-free basis, which gave 1034 mgm. of nitrogen per pot. The crop weights and the amounts of nitrogen in the crop are given separately as tops and roots.

TABLE 14

Nitrogen balance—Cowpeas I

Pot series 2, soil treated with clover tops

POT	NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	NITROGEN IN POT AT CLOSE	WEIGHT OF CROP		NITROGEN IN THE CROP		NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
					Tops	Roots	Tops	Roots				
	lbs.	*gm*	*mgm*	*mgm*	*gm*	*gm.*	*mgm*	*mgm*	*mgm.*	*mgm*	*mgm*	*mgm.*
1	None	9 7140[a]	3 5308[a]	1199[b]	21 70	6 976	604[c]	135[d]	1938	1006[e]	932	877
2	None	9 7324	3 4744	1178	19 65	6 040	559	126	1863	1041	822	
3	None	9 7423	3 3086	1120	18 75	7 170	504	157	1779	1045	734	749
4	None	9 7026	3 2257	1097	21 90	10 182	502	178	1777	1012	765	
11	995[f]	9 7421	6 8328	2314	34 75	11 705	1066	201	3581	2641	941	913
12	995	9 7419	7 0649	2393	35 30	9 525	1032	205	3630	2744	886	
13	1960	9 7324	9 5746	3246	33 00	10 550	977	192	4415	4206	209	234
14	1960	9 7412	9 5912	3249	39 75	12 711	1125	209	4576	431[illegible]	259	
15	2890	9 7462	11 5307	3904	39 20	11 959	1217	204	5325	5255	70	−81
16	2890	9 7514	11 6965	3958	36 45	12 270	1124	226	5308	5541	−223	
17	3790	9 7549	13 6027	4601	43 65	9 975	1256	153	6010	6786	−776	−632
18	3790	9 7468	13 4631	4558	37 25	8 347	1290	189	6037	6526	−489	
19	4660	9 7461	15 6151	5287	45 70	10 255	1594	125	7006	7875	−896	−927
20	4660	9 7516	15 9400	5394	41 45	8 215	1386	153	6933	7891	−958	

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[a] Average of three determinations.

[b] Calculated on the basis of 3300 gm of water-free soil.

[c] Found by analyzing three 2-gm samples and calculating from the weight of tops.

[d] Found by analyzing the entire root system.

[e] Based on analysis made at the close of the soybean series (data in table 10).

[f] The additions of nitrogen as clover tops made on these soils previous to the growth of soybeans are given as pounds of nitrogen added per 2,000,000 pounds of the mixture of soil and clover tops.

Following the tables is a graphical representation of the nitrogen balance in the series (fig. 2).

In the series treated with sodium nitrate the amounts of total nitrogen present at the close were larger in every pot than those present at the outset.

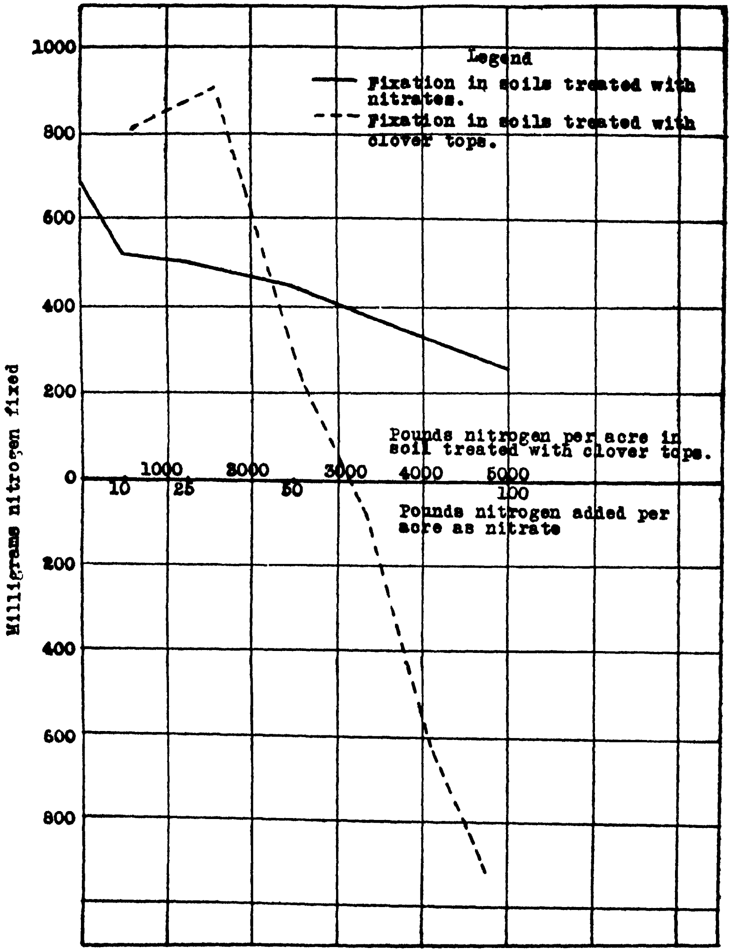

FIG. 2. GRAPHS SHOWING NITROGEN FIXATION BY COWPEAS ON SOILS TREATED WITH NITRATES, AND WITH CLOVER TOPS

This indicates decidedly that nitrogen was fixed from the atmosphere. The amounts so obtained were very significant, and in every case far above the limits of variation in analytical determinations. The average nitrogen increase, or nitrogen fixation, from duplicate pots shows a gradual decrease with increase in nitrate nitrogen added to the soil. The largest fixation appeared in the pots whose soil received no nitrogen additions and was reduced about the same in the two soils treated at the rate of 10 and 20 pounds of nitrate nitrogen per 2,000,000 pounds of soil. On the soil treated with the equivalent of 100 pounds of nitrate nitrogen the fixation was about 38 per cent of that where no treatment was applied. The general decrease in fixation with increased treatment would lead one to believe that one or two more increments beyond the application of 100 pounds would have prohibited fixation completely. The table indicates that increasing amounts of nitrate nitrogen in the soil reduce nitrogen fixation by cowpeas, and may perhaps prohibit it, but the amounts required for significant reduction of this process are far greater than ever occur in a soil or are ever applied.

The series treated with organic matter and previously used for growing soybeans had some pots which failed to show any increase in total nitrogen at the close over that at the outset. Significant increases were obtained for the check pots and those originally treated with the equivalent of 1000 and 2000 pounds of nitrogen per acre, but for the rest of the treatments a negative fixation, or loss, took place. This was greater with larger applications of nitrogen. It is highly probable that the organic matter put on the soil in such heavy applications one season previously was still undergoing decomposition rapidly enough to lose nitrogen as ammonia or as gaseous nitrogen. Decomposition had not yet gone far enough to change all the organic matter of such heavy applications into a more stable form, from which no losses could take place. Such losses from the total nitrogen present in soil and crop at the close, as compared with that present in soil and seed at the beginning, do not prove that the plant failed to draw on the atmosphere for some of its nitrogen supply. Nodules were plentiful in the soils receiving the heaviest applications of organic matter and some use may well have been made of gaseous nitrogen. Any fixation that could have taken place was offset by the losses from the soil, and could not be detected in this method of determination.

Considering the averages for duplicate pots, the amounts fixed in the check pots 1 and 2, which had previously grown the uninoculated soybeans, were larger than that fixed by pots 3 and 4, on which the soybeans were inoculated. This difference between the two sets of check pots resulted from a larger amount of nitrogen being present in both the crop and the soil in the first two pots. The reverse was true, however, of the total crop weights, largely because of greater root development in pots 3 and 4. In pots 11 and 12 the average fixation was the largest in the series. This would indicate that the treatment with organic nitrogen corresponding to 1000 pounds per acre was beneficial to nitrogen fixation by cowpeas in a soil so low in this element as this soil was. This statement cannot be made, however, for all the higher applications. A decided increase was shown by the pots receiving 2000 pounds of nitrogen per acre, but those receiving more showed a loss rather than a gain.

Evidently the lower applications do not prohibit nitrogen fixation, but whether the larger ones prohibit the process cannot be said from the preceding data. The losses coming doubtlessly from the decaying organic matter in the soil more than balance any nitrogen from the air added to the plant. In terms of total nitrogen in the soil rather than nitrogen added, those pots showed nitrogen fixation whose soils contained the equivalent of approximately 625, 1625 and 2600 pounds of nitrogen per 2,000,000 pounds. From this it may be safely said that a soil with these amounts of nitrogen does not prohibit the symbiotic nitrogen fixation even when 2000 pounds of nitrogen were in a readily decomposable form. The soils with higher nitrogen content failed to show similar results because the large amount of nitrogen applied to the soil was partly lost in a volatile form.

Series 3 (Cowpeas)

The third crop series was very similar to the second, with a few modifications. It included one series of pots with soils treated with nitrates and another series with soils treated some time previously with organic matter in the form of clover tops.

For treatment with nitrates some soil saved by careful storage from the first crop series of soybeans was used. Sodium nitrate was added in solution to increase the nitrogen content at increments equivalent to 50, 100, 150, 200 and 250 pounds of nitrogen per 2,000,000 pounds of soil. Two additional pots receiving the equivalent of 50 pounds of nitrate nitrogen were included, but this was added in three applications at intervals extending over the greater part of the growing period. Three kilograms of soil were used per pot.

For soils whose nitrogen was increased by organic matter, those pots used in both preceding crop series were used again without change, save that the amounts of soil were less, and only 2800 gm. of water-free soil were weighed into each pot. The nitrogen content of these soils did not increase in constant amounts for the series, but corresponded very closely to amounts equivalent to 625, 1625, 2600, 3250, 4025 and 4775 pounds of nitrogen per 2,000,000 pounds of soil These soils had been made up originally with enough clover tops to give a series whose nitrogen content increased by units of 1000 pounds, but the losses during the two preceding crop series reduced it to the above figures. It was thought advisable to use these soils again since their nitrogen content was accurately known, and the growth of two crops allowed sufficient time to permit decomposition of the organic matter to have gone far enough to prohibit further losses in volatile nitrogen.

The soils were treated with calcium carbonate at the rate of 2 tons per 2,000,000 pounds of soil. Phosphorus, potassium and other mineral plant-food elements were applied in solution when the dry soil was first moistened and then at intervals during the crop growth. All soils were treated similarly in this respect. Attempts were made to keep the moisture content of the soil at optimum by weighing the pots, but this method was inaccurate when the plants became larger, so that water was applied at intervals to keep the moisture content near what seemed optimum.

Cowpeas were grown as the crop. Six seeds weighing in total an average of 1.109 gm. and containing 38 mgm. of nitrogen were planted in each pot. As the seedlings were well started, one was removed to leave five vigorous plants.

The growth of the crop was good. No difficulty was experienced in germination, and though the early growth was somewhat slow, the remainder of the growing season found the plants doing well. Differences due to treatment were manifested early. With increased amounts of nitrate applied the plants were taller, of a deeper color and the leaves more fleshy, but these marked differences disappeared later. The plants on soils treated with organic matter were larger and of deeper color as the nitrogen content of the soil increased. These variations became less prominent and were completely lost before the close of the experiment. The total plant growth was similar to that in the preceding crop series, but with less variations. Plate 4 illustrates the crop growth of the two series just before harvest, which was about 22 weeks after planting.

Shortly before the crops were harvested, several blossoms were formed but were removed to keep these few plants from setting seed. All blossoms and fallen leaves were collected and saved for analysis. The harvested plants were dried in the greenhouse, and later weighed and thoroughly ground for analysis.

The roots were carefully removed, examined for nodules and thoroughly dried. No great differences in nodule production were evident as correlated with the treatment. There were no significant differences shown by the nodules in the soils treated with nitrates. Nodules were numerous in all pots and especially so in those receiving the equivalent of 250 pounds of nitrogen per 2,000,000 pounds of soil. There was no evidence in either of the duplicate pots that the application of such large amounts of nitrate interfered with nodule production. This application was equivalent to 12.5 mgm. of nitrogen per 100 gm. of soil and its failure to suppress nodule growth agrees well with the results of Fred and Graul (1[illegible]) who found that 10 mgm. of nitrate nitrogen per 100 gm. of Miami silt loam were required before nodules of alfalfa and crimson clover were decreased by such treatment. Evidently the application in the yellow silt loam used in this case was still less than was required for serious effect on nodule production.

Nodules were present in all pots treated with organic matter and gave indications of being larger and better distributed within those soils receiving the heaviest applications This may have been due to the better physical condition and greater aeration caused by such treatment. These results are not in accord with those found by Frank (14) for red clover, on which the nodules were fewer and smaller in the soil rich in organic matter.

At the close of this series, determinations of nitrate nitrogen were made on all the soils. Those treated with nitrate failed to show any nitrogen of this form present in the soil. This result is quite different from that found for soybeans, in which case an application of 258 mgm. per pot left much nitrate in the soil. The total nitrogen in the crop of soybeans was only 150 mgm. With the profuse growth of cowpeas in this series the application of 390 mgm. of nitrogen as nitrate was far below the nitrogen in the crop and might well be expected to be removed on account of its solubility. The removal was so complete that no more than a trace of nitrate was found in any of the soils.

TABLE 15

Nitrogen balance—Cowpeas II

Pot series 1, soil treated with nitrates

POT	NITRATE NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	NITROGEN IN POT AT CLOSE	WEIGHT OF CROP		NITROGEN IN THE CROP		NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
					Tops	Roots	Tops	Roots				
	mgm	gm	mgm.	mgm	gm	gm.	mgm.	mgm	mgm	mgm	mgm.	mgm.
1b	None	9 7363[a]	3 7909[a]	1168[b]	32 30	10 230	778[c]	153	2099	965[d]	1134	1212
2b	None	9 7455	3 8321	1179	36 35	11 740	909	168	2256	965	1291	
3b	75	9 7396	3 8543	1187	34 53	8 527	884	146	2217	1040	1177	1125
4b	75	9 7428	3 7909	1167	31 55	9 196	800	147	2114	1040	1074	
5b	150	9 7419	3 7223	1146	33 88	10 374	828	177	2151	1115	1036	1248
6b	150	9 7414	4 1902	1290	41 23	14 539	1109	177[e]	2576	1115	1461	
7b	225	9 7477	3 7863	1165	36 38	12 709	904	196	2265	1190	1075	1185
8b	225	9 7547	4 0892	1257	38 03	9 569	1064	165	2486	1190	1296	
9b	315	9 7486	3 9412	1212	37 08	10 756	968	175	2355	1280	1075	1092
10b	315	9 7535	4 1229	1268	36 78	12.215	929	192	2389	1280	1109	
11b	390	9 7453	3 9108	1203	38 53	9 831	1130	159	2492	1355	1137	1187
12b	390	9 7444	4 0892	1258	37 28	9 509	1138	197	2593	1355	1238	
13b	75[f]	9 7354	3 8368	1182	22 83	11 868	559	179	1920	1040	880	935
14b	75	9 7447	3 9850	1226	27 41	10 411	651	153	2030	1040	990	

[a] Average of three determinations

[b] Calculated on the basis of 3000 gm of water-free soil.

[c] Found by analyzing three 2-gm samples and calculating from the weight of tops.

[d] Calculated on the basis of 3000 gm. of water-free soil with 0 0311 per cent of nitrogen, or 933 mgm of nitrogen, and five cowpea seeds with 32 mgm. of nitrogen.

[e] Part of roots lost, and duplicate pot was used for data.

[f] Applied as three applications at intervals during the growth of the crop.

For the organic matter series about the same conditions obtained. No significant amounts of nitrates were present in any pots and only traces were found in those which had originally received the equivalent of 5000 pounds of nitrogen per acre as clover tops. Evidently the organic matter had undergone sufficient changes so that it no longer contained volatile organic compounds which interfered with the, reduction method employed for determining nitrates. Nitrification may have been going on but not at sufficient rates to permit measurable accumulations above the consumption by the plants. The rapid growth of the plants might well have removed all the nitrates formed by rapid nitrification processes. Since the determinations failed to show nitrates present, no tabulations of data are submitted.

To determine the total nitrogen in the crop and soil at the close, the procedure of the previous series was followed. All possible precaution was exercised to eliminate errors and maintain uniform results throughout. Tables 15 and 16 give the data of this series, showing the increase in total nitrogen at the close over that present at the beginning.

TABLE 16

Nitrogen balance—Cowpeas II

Pot series 2, soil treated with clover tops

POT	NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	NITROGEN IN POT AT CLOSE	WEIGHT OF CROP		NITROGEN IN THE CROP		NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
					Tops	Roots	Tops	Roots				
	lbs	*gm.*	*mgm*	*mgm*	*gm.*	*gm*	*mgm*	*mgm.*	*mgm*	*mgm.*	*mgm*	*mgm.*
1	None	9.7280[a]	4 1271[a]	1187[b]	34.58	6 886	901[c]	136[d]	2224	1049[e]	1175	1138
2	None	9 7293	4.0034	1152	32 40	10 846	810	169	2131	1030	1101	
3	None	9 7392	3 7909	1089	27 20	9 466	619	162	1870	982	888	873
4	None	9 7321	3 7020	1064	25 00	8 044	607	148	1819	961	858	
11	995[f]	9 7427	7 2487	2083	35 00	6 378	1037	137	3257	1995	1262	1292
12	995	9 7498	7 4031	2126	39 55	5 951	1127	131	3384	2062	1322	
13	1960	9 7454	9 7580	2803	36 55	7 379	1160	145	4108	2786	1322	1295
14	1960	9 7398	9 7739	2809	39 40	6 727	1125	122	4056	2788	1268	
15	2890	9 7481	11 9310	3427	36 93	6 481	1039	124	4590	3344	1246	1261
16	2890	9 7576	11 8676	3405	33 93	6 014	1152	109	4666	3390	1276	
17	3790	9 7543	14 1009	4047	35 75	6 666	1012	117	5176	3946	1230	1215
18	3790	9 7608	13 3840	3839	38 26	8 482	1130	131	5100	3900	1200	
19	4660	9 7597	15 8616	4550	32 61	9 492	927	132	5629	4517	1112	1155
20	4660	9 7553	15 7601	4523	39 28	8 595	1136	148	5807	4609	1198	

[a] Average of three determinations.

[b] Calculated on the basis of 2800 gm. of water-free soil

[c] Found by analyzing three 2-gm. samples and calculated from weight of tops.

[d] Found by analyzing entire root system.

[e] Based on analyses made at the close of the preceding cowpea series. Data in table 14 figured for 2800 gm. of water-free soil and five cowpea seeds with 32 mgm. of nitrogen.

[f] The additions of nitrogen as clover tops made on these soils previous to the growth of soybeans are given as pounds of nitrogen added per 2,000,000 pounds of the mixture of soil and clover tops.

The above data show most decidedly that the addition of nitrates in even larger amounts than were used in the previous series does not prohibit an increase of nitrogen fixation. The use of this form of nitrogen in the previous test with cowpeas gave a gradual decrease of fixation as the application of nitrate increased. In that series nitrate equivalent to 100 pounds per 2,000,000 pounds of soil permitted only 38 per cent as much fixation as no treatment, hence it suggested that double the application would prohibit fixation completely. In this series the applications were more than doubled and increased to as much as 250 pounds of nitrogen per 2,000,000 pounds of soil, yet no serious hindrance was given to nitrogen fixation. The outstanding characteristic of the data is the lack of variations in nitrogen fixed. The average figures for duplicate pots show a fixation of approximately 1200 mgm. for all pots except the two receiving 315 mgm. of nitrate nitrogen at the outset, and the two receiving 75 mgm. in three successive applications. Those pots with 390 mgm. of added nitrogen gave a fixation of 1187 mgm., corresponding closely to the fixation of 1124 and 1212 mgm. in the check pots. The data indicate that these amounts of nitrate nitrogen used in this series had no significant effect either favorable or unfavorable, and the highest treatment gave no significant variation from the general uniformity of the series.

In pots 13b and 14b receiving three applications of 25 mgm. each or a total equivalent to 50 pounds of nitrogen per 2,000,000 pounds of soil, the fixation was the lowest in the series. The crops on these pots were the smallest of all pots, as is shown by the weights. The roots were about as heavy in these pots as any in the series, but the tops weighed far less than those of any other treatments. The same relations held for the total nitrogen in the tops and roots of these two pots. That of the tops was less, and that in the roots was about the equal of those of the other treatments of the series.

The fact that the fixation in pots 13b and 14b was not equal to that of 3b and 4b might lead one to believe that the application of 50 pounds of nitrate at the time the seed was planted gave more fixation than this same amount of nitrogen distributed over three applications during the plants' growth. The soluble nitrogen applied early may have enabled the plant to establish itself better at the outset and fix more nitrogen than when the same amount of soluble nitrogen was put into the soil at intervals. Pots 3b and 4b were treated exactly the same as pots 13b and 14b, save that the latter two did not receive all the nitrogen at the beginning, and such conclusions might be drawn from the comparison alone. When both these treatments are compared with the check, however, the interpretation must be different. The fixation when all the nitrates were applied at the outset was no larger than that of the check receiving no treatment, and was less than that of the check when applied at intervals during the plants' growth. On this basis the application of all nitrogen at the beginning showed no influence on the fixation, while the application at intervals kept it down

The series with nitrates gives large amounts of nitrogen fixation for all pots, but little variation caused by treatments. It would indicate that nitrates used in amounts as high as 1500 pounds per acre do not exert any effect, unless perhaps when the applications are made at intervals during the plants' growth rather than at the beginning.

The second set of pots, whose total nitrogen content of the soil increased by approximately 1000 pounds per acre, showed results quite different from those it gave in its previous crop of cowpeas. Instead of losses of nitrogen, there were marked gains in all pots, indicating that the decaying organic matter had no longer lost nitrogen and that the nitrogen taken from the air appeared as increase in the total determinations at the close of the series. Check pots 3 and 4 whose nitrogen in the soil and crop was lower than that in check pots 1 and 2 in the previous crop of cowpeas, showed similar results again. The differences in the soil nitrogen of these two sets of checks amounted to more than 100 mgm. and in the crop they were even greater, thus giving a difference of over 250 mgm. in the amounts of nitrogen fixed by the two sets of checks. The use of one or the other set of checks as a basis for comparison will influence the interpretation of the results of the treatments, but only in degree rather than in kind, since all the treatments gave results above even the highest of the checks. The results from the four check pots were used as a basis in calculating the increase, or nitrogen fixation, as influenced by treatment.

By using the average of the four check pots as a basis for comparison all the treatments with organic matter increased the fixation of nitrogen. For those pots receiving the heaviest applications the increase is not as large as in the others, but all pots below this addition of 5000 pounds of nitrogen per acre gave a significant increase in fixation over even the highest of the check pots. This indicates that the addition of the organic matter increased the fixation, rather than hindered it, and that the soils richer in total nitrogen permitted the plant to draw on the atmospheric supply of this element as well as on that in soil. These facts are not in accord with the general statements often made, that the plant fails to draw on the nitrogen of the atmosphere until forced to do so by the deficiency in the soil. Here the results were the very reverse and the addition of organic nitrogen enabled the plants to take more nitrogen from the air as a result of such treatment. Such benefit was suggested even in the two previous crops, where the addition of 1000 pounds of nitrogen as organic matter gave in every case greater fixation than in soils not so treated. According to these data 1000 pounds of nitrogen added as organic matter increased nitrogen fixation as much as the larger applications used in the series and the fixation decreased slightly with increased additions, but this decrease was so small as to be insignificant. Even if this decrease were larger there would be no cause for alarm since the application of 1000 pounds of nitrogen as organic matter is equivalent to approximately 25 tons of clover tops and is far greater than is ever applied to soils for fertilizer uses, and such amounts of readily decomposable nitrogenous materials seldom or never occur in common soils.

The graphic representation of the data in figure 3 shows that cowpeas growing on soils treated with nitrates, or rich in organic matter may utilize atmospheric nitrogen even when the amounts of these forms of nitrogen are very large. They show further, that neither the nitrate treatment nor the soil's high total nitrogen content affect nitrogen fixation detrimentally, but rather, that the treatment with organic matter favors the process by increasing the amount of atmospheric nitrogen taken by this legume.

The total nitrogen content in the soil of the one series was equivalent to 775, 1500, 2000, 2440, 2800 and 3240 pounds in 2,000,000 pounds, while the fixations for these were 1005, 1292, 1295, 1261, 1215 and 1155 mgm. per pot, respectively. The increases in fixation caused by the organic matter over that of the check are 287, 290, 256, 210 and 150 mgm. with the increasing nitrogen content of the soil. Accordingly, the soils richer in nitrogen gave somewhat

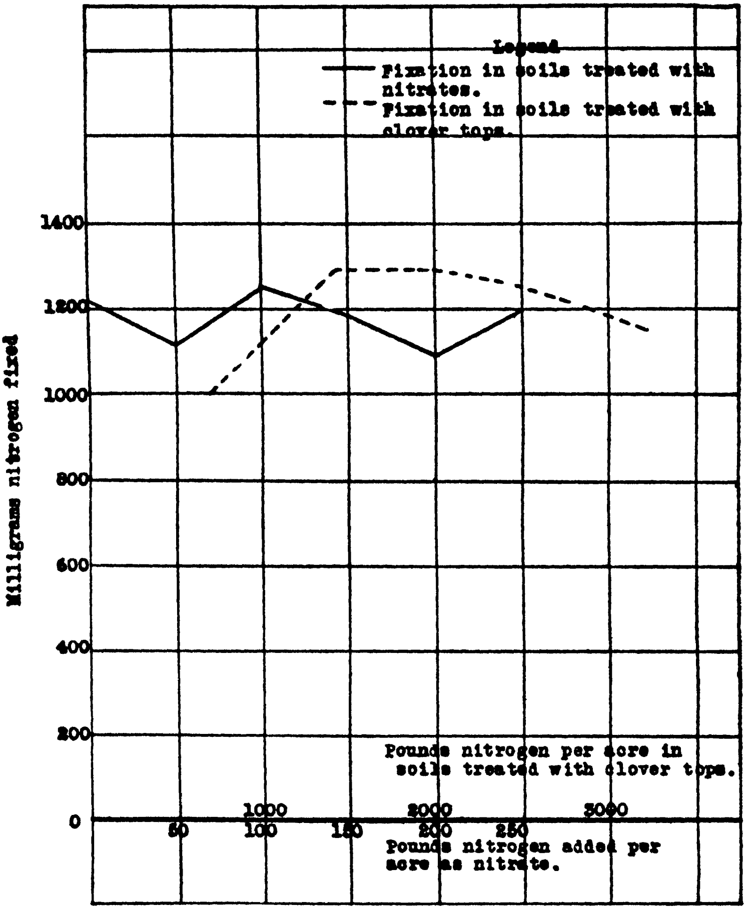

FIG. 3. GRAPHS SHOWING NITROGEN FIXATION BY COWPEAS ON SOILS TREATED WITH NITRATES, AND WITH CLOVER TOPS

less fixation than the soils poorer in this respect, but these differences are not significant when compared with the total nitrogen fixed. It may be possible that the higher treatments still lost some nitrogen from the soil during this time, and if proper corrections could be made they might show the same fixation throughout the series.

Nitrogen changes in soils during the growth of legumes

Since the soils used in this experiment were carefully analyzed for each crop grown on them, they offer an excellent opportunity for study of the changes in the nitrogen taking place within the soils themselves.

The soils in pots 1 to 4 and 11 to 20 were used during the entire series of three successive crops. The roots were removed as completely as possible to prevent addition of organic matter in this form. As these soils received applications of larger amounts of clover tops at the outset, they offer an interesting study of the changes in nitrogen content while this organic matter was decaying, and while three successive crops of legumes were growing. The following data are tabulated to give the changes in the nitrogen content of these soils as shown at the close of each crop. In the treated soils, from which there were losses of nitrogen, some estimate of these losses can be made, while in the untreated soil some measure may be taken of the influence of the legume plant on the nitrogen content of the soil itself. The figures were obtained from the analyses of the soil and organic matter at the beginning and from that of the soil alone at the close of each crop series. For the sake of uniformity the data are tabulated as pounds per acre of 2,000,000 pounds of soil. The data are given in table 17.

For the soils treated with organic matter, the losses rather than gains in nitrogen are the chief characteristics. During the growth of the soybeans no losses took place in the soils treated with less than 2890 pounds of nitrogen per acre. For this application the average loss was 242 pounds. For the two higher treatments the average losses were 382 and 510 pounds.

During the growth of the second crop, cowpeas, there were losses in all pots, and all were greater than occurred in these same soils during the first crop. The lowest two treatments, which had lost no nitrogen previously, showed decreases equivalent to 205 and 614 pounds per acre, while in those pots with larger applications of organic matter, the nitrogen losses were 892, 1254 and 1539 pounds. In terms of the original amounts of nitrogen applied these losses correspond to 20.6, 30.3, 33.0, and 33.0 per cent, respectively. Evidently the most rapid decomposition and heaviest losses did not take place until the time of the second-crop growth, which closed 201 days after the soils were treated. Drying and grinding the soil between the two crops may have hastened decomposition.

For the period of time in which the third crop was grown the data show results different from those for the two preceding periods. The treated soils no longer gave losses of nitrogen, but in all cases gave gains for this element. The largest average gain was 77 pounds in the pots receiving 995 pounds of nitrogen per acre, but in the other pots the gains decreased with increased treatments, though in no regular order. These increases in the soil nitrogen are not large, but big enough to be considered as increases as measured by accurate analytical determinations varying less than 0.2 mgm. of nitrogen in 10-gm. samples of soil in triplicate determinations. This range in difference corresponds to 40 pounds per acre, and the error becomes less when three samples are taken. Even though these increases were not considered, there is no small importance in the fact that these soils produced such heavy crops of legumes and yet suffered no loss in nitrogen. In some cases the nitrogen in the total crop was over half as much as that originally in the soil. Had the crop taken its nitrogen from the soil, such amounts removed could easily have been measured by ordinary analytical methods.

TABLE 17

Changes during the growth of three crops of legumes in a soil treated with organic matter

All figures given in pounds per 2,000,000 pounds of soil

POT	CLOVER TOPS ADDED	NITROGEN ADDED	NITROGEN IN THE SOIL						
			At the beginning	After first crop (soybeans)	Increase	After second crop (cowpeas)	Increase	After third crop (cowpeas)	Increase
	lbs	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs.*	*lbs.*
1	None	None	625	583	−18	726	113	847	115
2	None	None	625	630		713		822	
3	None	None	625	633	−2	678	48	778	98
4	None	None	625	613		664		760	
11	37,766	995	1620	1600	11	1402	−205	1488	77
12	37,766	995	1620	1663		1450		1518	
13	75,532	1960	2585	2550	−2	1967	−615	2002	35
14	75,532	1960	2585	2615		1968		2006	
15	113,298	2890	3515	3185	−242	2365	−892	2448	60
16	113,298	2890	3515	3360		2395		2432	
17	151,064	3790	4415	4110	−382	2795	−1254	2890	37
18	151,064	3790	4415	3955		2762		2742	
19	188,830	4660	5285	4770	−510	3203	−1538	3250	4
20	188,830	4660	5285	4780		3269		3230	

The greatest increases in nitrogen of the soil occurred in the four check pots which had been left untreated. During the growth of the soybeans these soils showed no significant change, save a very slight loss. During the next crop, which was cowpeas, they showed decided gains in nitrogen, and again gave similar results for the last crop of this same legume. The soils in all four pots increased in nitrogen by growing the cowpeas. It cannot be specifically said whether this was due to a direct change in the original soil material itself, or to the addition of small roots and nodules that remained in spite of the careful attempt to remove them; but it seems most probable that the latter reason was sufficient to give the increases recorded. With such gains in soil nitrogen when the roots are removed, surely the incorporation of the roots themselves would give a decided increase. It has been said for some legumes (25, p. 218) that the addition of merely the roots does not increase the nitrogen content of the soil, but the above data indicate that the 625 pounds of nitrogen originally present in this soil increased when the roots were removed as carefully as possible, and would have increased much more if the roots had been included. The same may be said of the richer soils during the last crop of cowpeas, all of which showed some gains in nitrogen content when the roots were removed. Had the roots been left in the soil its nitrogen content would have been raised decidedly, even where the total nitrogen of the soil was already equal to 3200 pounds per acre. None of the soils in the last crop series, whether high or low in nitrogen, lost in this element as the result of the removal of the entire crop of roots and tops of the plants. All would have been enriched in this respect had only the roots been added.

Distribution of nitrogen in roots and tops of cowpeas

It has been known that the portions of the total plant substance which are in the tops and in the roots are not constant for the different kinds of plants, nor are they always constant for the same kind of plant (24, 40, 49). Similar statements may be made concerning the distribution of the constituent elements of the plant tissue, especially the nitrogen. The data of this experiment were taken in the hope of studying the distribution of the nitrogen in the tops and roots, as well as the weight of these plant parts as influenced by the nitrogen content of the soil. The plants were harvested by cutting the stems just level with the soil surface, and considering as tops that part above the soil level, and as roots all parts below it. Only the two cowpea crops were treated in this manner and the data are given for this legume grown in a soil with varying amounts of nitrates and in a soil containing varying amounts of organic nitrogen. The data were taken from the previous table and are given as averages of the duplicate pots for each treatment (tables 18 and 19). The distributions of the weights and nitrogen in the tops and roots are expressed as per cent of the total.

According to the data, the addition of nitrate nitrogen tends to increase the weight of the plant top faster than that of the roots. In the first cowpea crop, the percentage of the total plant weight in the tops increased gradually while that in the roots decreased as the amount of nitrate nitrogen added became larger. In the second crop there was also an increase, but not such a marked one. The entire second series showed a higher percentage of the total plant weight in the top than was shown in the previous series. Evidently some other factor was coupled with the nitrogen in the soil to influence the distribution of the weights in tops and roots. This may well be expected since the

TABLE 18

Distribution of nitrogen in the roots and tops of cowpeas

Soil treated with nitrates

NITROGEN ADDED PER POT	PART OF PLANT	WEIGHT	PER CENT OF TOTAL	TOTAL NITROGEN	PER CENT OF TOTAL
		First crop			
mgm		*gm.*		*mgm*	
None	Tops	15.25	62.51	416	72.40
	Roots	9.145	37.48	158	27.52
16.5	Tops	15.60	66.63	373	71.45
	Roots	7.810	33.36	149	28.54
41.0	Tops	16.05	68.26	368	68.91
	Roots	7.463	31.73	166	31.08
82.0	Tops	16.40	67.09	424	71.99
	Roots	8.043	32.90	165	28.00
157.0	Tops	15.125	69.86	366	73.34
	Roots	6.704	30.71	131	26.25
		Second crop			
None	Tops	34.32	75.75	843	84.04
	Roots	10.985	24.24	160	15.95
75.0	Tops	33.04	78.85	841	85.20
	Roots	8.861	21.14	146	14.79
150.0	Tops	37.55	75.09	968	84.54
	Roots	12.456	24.90	177	15.45
225.0	Tops	37.20	76.95	984	84.53
	Roots	11.139	23.04	180	15.46
315.0	Tops	36.93	76.27	948	83.81
	Roots	11.485	23.72	183	16.18
390.0	Tops	37.90	79.61	1134	86.43
	Roots	9.705	20.38	178	13.56
75.0	Tops	25.12	69.27	605	78.46
	Roots	11.139	30.72	166	21.53

TABLE 19

Distribution of nitrogen in the roots and tops of cowpeas

Soil treated with clover tops

NITROGEN IN SOIL	PART OF PLANT	WEIGHT	PER CENT OF TOTAL	TOTAL NITROGEN	PER CENT OF TOTAL
		First crop			
lbs [a]		*gm*		*mgm*	
None	Tops	20.67	76.05	581	81.71
	Roots	6.508	23.94	130	18.28
None	Tops	20.32	70.07	503	75.07
	Roots	8.676	29.92	167	24.92
995	Tops	34.92	76.68	1049	83.78
	Roots	10.615	23.31	203	16.21
1960	Tops	36.37	75.77	1051	84.21
	Roots	11.630	24.22	197	15.78
2890	Tops	37.82	75.73	1170	84.47
	Roots	12.114	24.26	215	15.52
3790	Tops	40.45	81.53	1273	88.15
	Roots	9.161	18.46	171	11.84
4660	Tops	43.57	82.51	1490	91.46
	Roots	9.235	17.48	139	8.53
		Second crop			
719	Tops	33.49	79.06	855	84.90
	Roots	8.866	20.91	152	15.09
621	Tops	26.10	74.88	613	79.81
	Roots	8.755	25.11	155	20.18
1426	Tops	37.77	85.96	1082	88.98
	Roots	6.164	14.03	134	11.01
1967	Tops	37.97	84.33	1142	89.56
	Roots	7.053	15.66	133	10.43
2381	Tops	35.43	85.01	1095	90.42
	Roots	6.247	14.98	116	9.57
2778	Tops	37.00	83.00	1071	89.62
	Roots	7.574	16.99	124	10.37
3236	Tops	35.94	79.89	1031	87.29
	Roots	9.043	20.18	150	12.70

[a] Pounds in 2,000,000 pounds of soil.

crops were grown at different seasons. Nevertheless, the nitrogen in this readily soluble form was of influence, and increased the plant growth above the ground more than it did the development of the root system.

In the soils with varying amounts of organic nitrogen, the higher nitrogen content of the soil gave a greater portion of the plant weight in the tops, but there was no regular increase in this proportion with the regular increase in the soil nitrogen. This failed to show the uniformity of increase shown by the soils treated with nitrates, but yet indicated that as the soils were richer in nitrogen, the weight of the plant above ground increased faster than the weight of the roots.

Considering the distribution of the total nitrogen of the plant, the effects of the nitrates of the soil toward increasing the portion of this in the tops are not as marked as for increasing the weight of the tops. The data of both series fail to show any regular influence, by the nitrates, on the percentage of the total plant nitrogen found in the roots or the tops. The effect of the organic nitrogen in the soil was shown as a more rapid increase in the nitrogen of the tops, than that of the roots. In the first crop this effect was very decided, giving 10 per cent more of the plant's total nitrogen in the tops for the soil with high nitrogen content than for the soil to which no organic nitrogen had been added. In the second crop, all the soils richer in nitrogen than the untreated soil had a bigger portion of the plant's nitrogen in the tops, but there was no close correlation between the soil treatment and the disturbance in the distribution of the total nitrogen in the plant tops and roots. Nevertheless, it is evident that the nitrogen in the part of the plant above ground increased with the treatment of both nitrates and organic matter.

Between the distribution of the nitrogen, and the fixation of this element, there is no correlation in this experiment. It cannot be said that the fixation is greater with increased production of that part above the ground, which is the relation that one might expect according to Peterman (41). Scarcely enough data were secured to warrant a specific statement on this question of relation of crop size to the amount of nitrogen fixation.

IV. SUMMARY

The experiment herein reported was a study of nitrogen fixation as influenced by the nitrogen content of the soil. Variations in the nitrogen of the soil were brought about by adding sodium nitrate, and by incorporating organic matter in the form of clover tops in a soil containing only 625 pounds of nitrogen per 2,000,000 pounds of soil. This gave variations in mineral nitrogen and organic nitrogen, respectively.

One crop of soybeans and two crops of cowpeas were grown. Some of the original soil was used each time for the treatment with nitrates, but the same soils were used throughout the three crops for the treatment with organic matter.

Nitrogen fixation is represented as the increase in the total nitrogen in the soil and crop at the close of the experiment, over that present in the soil and seed at the beginning.

The decaying organic matter occasioned some heavy losses in nitrogen from the soil. These losses reached the maximum during the second crop, and had apparently disappeared after 200 days. During the second crop, the highest losses of nitrogen from the soil were equivalent to 1538 pounds per 2,000,000 pounds of soil where the original application of clover tops was equivalent to 4660 pounds of nitrogen per acre. These losses from the soil with the heavy applications of organic matter made it impossible in a few instances to measure the nitrogen fixation. In those soils, however, to which were added nitrates and smaller quantities of organic matter, nitrogen fixation was easily measured and a decided amount was found.

The addition of the approximate equivalent of 1000 and 2000 pounds of nitrogen as organic matter per acre, corresponding to 18 and 36 tons of clover tops, respectively, gave nitrogen fixation for all three crops grown. For the last crop grown, during which time the soils no longer showed losses of nitrogen, there were decided gains, or nitrogen fixation, for all treatments with organic matter.

Nitrates did not prohibit nitrogen fixation. Indications in one series of cowpeas suggested that increasing amounts of nitrogen applied as sodium nitrate lessened the amount of fixation. A later series of the same crop with higher and wider ranges of the application of nitrates failed to show the same results, even when as much as 250 pounds of nitrogen was applied in this form. This corresponded to an application of more than 1500 pounds of sodium nitrate per acre.

The addition of sodium nitrate to the soil caused the plants to grow better at the beginning, but gave no increased nitrogen fixation for two series of cowpeas, save in one treatment of 150 pounds of nitrogen per acre, where the fixation was slightly greater than that of the check. Fixation in all other treatments with nitrates was less than that of the check.

After the soils treated with organic matter ceased to lose nitrogen by decomposition, they showed a large nitrogen fixation. The organic matter added caused this fixation to be larger than that in the soils not so treated. The increase in the nitrogen fixed as caused by the organic matter, was not proportional, however, to the amount of organic matter applied.

There were some variations in the amounts of nitrogen taken from the air by five plants of cowpeas in each pot. The maximum average fixation for duplicate pots was 1295 mgm. on a soil containing the equivalent of 2000 pounds of total nitrogen per acre. Amounts almost as large were obtained in soils treated with 150 pounds of nitrate nitrogen per acre.

Nodule production was not suppressed to an appreciable extent by any of the treatments of nitrate or organic matter.

The untreated soils which grew legumes for three successive crops showed gains in their nitrogen content as a result of these crop growths, even though all roots were removed from the soil as completely as possible. In the last crop of cowpeas grown, all soils from which the roots had been removed, became richer in nitrogen under this treatment, even though some of these soils contained more than 3000 pounds of nitrogen per acre before the crop was grown, and the crop itself contained more than one-fourth of this amount. This fact indicates that the growth of a legume may be large, but yet leave the nitrogen content of the soil nearly constant or even increase it, and that the addition of the plant roots will increase the soil's nitrogen content when there is no loss by leaching.

As the soil's content of total nitrogen or of nitrate nitrogen was higher, the yield of plant tissue became greater; and the greater part of this increase was in the tops of the plant rather than in the roots. With a higher nitrogen content of the soil, a bigger share of the plant's total nitrogen was also in the tops.

V. CONCLUSIONS

1. The results of this study indicate that nitrogen fixation will take place in a soil containing large amounts of nitrogen in the form of either nitrates or organic matter.
2. No injurious effects on nitrogen fixation were caused by nitrates in this experiment, and if such ever occur under conditions similar to those which obtained in this study, the application of nitrates must be many times larger than is ever applied in agricultural practice.
3. Nodules are produced when large amounts of organic nitrogen are present in the soil, and good legume growth results even when sufficient organic matter is present to give large losses of volatile nitrogen from the soils.
4. The addition of some organic matter may increase the amount of nitrogen fixed by cowpeas.
5. In soils containing varying amounts of total nitrogen, as much fixation of nitrogen by cowpeas may be expected in one with 3000 pounds of total nitrogen, as in one with lesser amounts. According to the data given, variations in the amount of total nitrogen in a soil failed to exert any varying influence on the amount of nitrogen fixed.

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This opportunity is taken by the author to express his obligations to Professor A. L. Whiting, of the Division of Soil Biology, under whose able direction this study was conducted.

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PLATE 1

SOYBEANS ON SOIL TREATED WITH NITRATES, CLOVER TOPS, AND WITH CLOVER TOPS AND NITRATES

PLATE I

Check
NaNO₃ 80 lbs N
Silt Loam
Check
Clover Tops
NaNO₃
Clover Tops
4000 lbs
Clover Tops
Clover Tops
Check
Clover Tops
Clover Tops
Clover Tops

PLATE 2

Cowpeas, at 50 Days, on Soil Treated with Nitrates (Above) and Cowpeas as the Second Crop on Soil Treated with Clover Tops (Below)

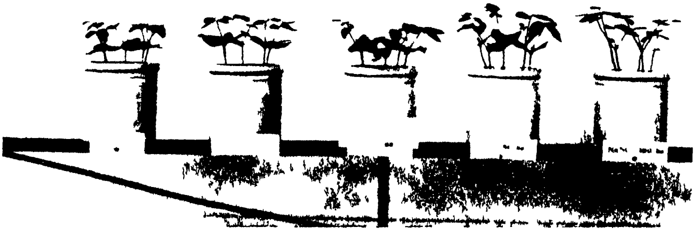


PLATE 3

COWPEAS AT 75 DAYS ON SOIL TREATED WITH NITRATES (ABOVE) AND COWPEAS AS THE SECOND CROP ON SOIL TREATED WITH CLOVER TOPS (BELOW)

PLATE 3

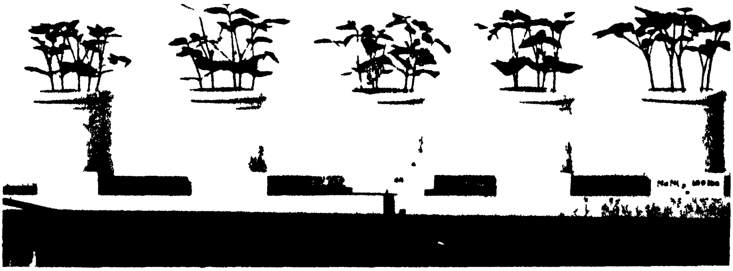
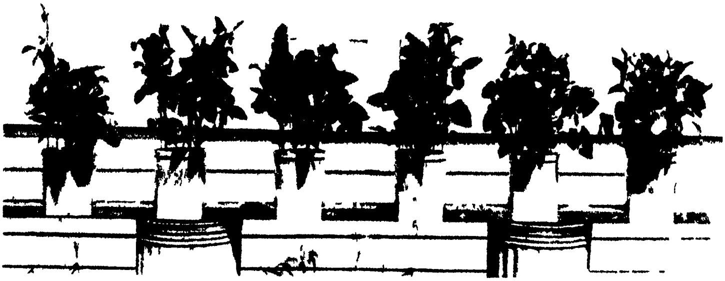


PLATE 4

COWPEAS ON SOIL TREATED WITH NITRATES (ABOVE) AND COWPEAS AS THE THIRD CROP ON SOIL TREATED WITH CLOVER TOPS (BELOW)



THE POTENTIAL BIOCHEMICAL ACTIVITY OF THE SPORES OF SOIL BACTERIA[1]

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INTRODUCTION

The microörganic population of an arable soil must accommodate itself to great fluctuations in moisture, temperature, aeration and food supply. Obviously only those types survive from season to season which are able to live during the unfavorable periods. Applications of manure and of decaying organic matter will introduce non-spore-forming organisms which may be active while there is sufficient food and moisture, but tend to disappear during subsequent exposure to desiccation. Conn and Bright (3, p. 325) found that non-spore-formers were only occasionally isolated from a soil which had not been manured for some time, while the spore-formers were always present in fairly constant numbers.

Thus it seems evident that considerable of the work of liberating plant-food from plant residues and from dried organic matter previously added, is performed under optimum moisture conditions and by organisms which previously existed as resting bodies or spores. In order to measure the potential biochemical activity as well as to study the distribution of spores existing at any given time, laboratory tests were conducted with heated and unheated infusions of fresh soil. These samples were taken at intervals during 1916 and a part of 1917, and were obtained to a depth of $\frac{2}{3}$ meter on two distinctly different types of arable soil.

It is hard to determine the amount of heat which should be applied to an infusion as cosmopolitan as that of the soil in order to destroy the vegetative cells and as few as possible of the spores. Lipman (6, p. 230) used a temperature of 80°C. for 10 minutes and found that infusions thus heated and inoculated into sterile peptone solution produced nearly as much ammonia at the end of 4 days as the unheated infusions. Conn (2, p. 188) used a temperature of 75°C. for 15 to 20 minutes, and found that the numbers of spore-formers appearing on gelatine plates was nearly as great with the heated as with the unheated inoculum. He also obtained no increase in the numbers of spore-formers as a result of adding heavy applications of manure to a soil and concluded that sporulating bacteria of the soil multiply but slightly and exist for the most part as inactive spores.

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Hiss and Zinsser (4) state that heating to 55° or 60°C. for 10 minutes causes the death of most vegetative forms. Lauback (5, p. 493) used a temperature of 90°C. for 15 minutes for his isolation of non-pathogenic bacterial spores. Meyer (7, p. 127) studied the thermal death-points of the spores of various pure cultures and found that the most sensitive could withstand a temperature of 80°C. for nearly 3 hours.

The infusions used in this work were heated to 85°C. for 10 minutes. This temperature undoubtedly destroyed some of the spores as well as the vegetative cells, but it also destroyed nearly all of the fungi and all of the actinomycetes and chromogenic bacteria. At least these organisms failed to appear on the plates inoculated with the heated infusions, but were numerous on those inoculated with unheated infusions.

METHODS

The soil samples were obtained to a depth of 64 cm. at intervals of about 2 months by means of a bacteriological soil sampler previously described (8, p. 109). With the aid of this sampler, it was possible to secure each of the five samples from a small area without affecting the soil from which the following samples were to be taken. The samples were placed in sterile fruit jars in the field and infusions were obtained from them the same day. The infusions were prepared by weighing 50 gm. of the fresh soil into Erlenmeyer flasks containing 150 cc. of sterile water. These were shaken vigorously for 5 minutes and allowed to settle, after which portions were pipetted off for the heated and unheated inoculations. The portions to be heated were pipetted into selected thin-walled test-tubes. By means of rubber bands these tubes were held in place against the sides of a wire basket which was then placed in a large dish of water heated to a temperature of 87°C. The insertion of the basket and tubes would bring the temperature down to 85°C., where it was maintained. A control test-tube containing a thermometer indicated when the infusions had reached a temperature of 85°C. The water surface around the outside of the test-tubes was always higher than that within and the basket was moved about sufficiently to keep the liquids within the tubes in circulation. At the end of the 10-minute period, the tubes were withdrawn and allowed to cool.

Dilutions were made from all infusions thus prepared for the determination of numbers for which Brown's egg albumin agar (1, p. 381) was used. Counts were made after incubating the plates at from 20° to 22°C. for 8 days.

For the ammonia and carbon-dioxide determinations, 250-cc. Erlenmeyer flasks containing 70 gm. of soil, 100 mgm. of nitrogen in dried blood and water equivalent to 50 per cent of the maximum capacity of the soil, were sterilized at a pressure of one atmosphere for 30 minutes. These flasks were inoculated with 5 cc. of soil infusion and incubated for 7 days at from 20° to 22°C. Uninoculated flasks were incubated in several cases, but gave no ammonia accumulation over that originally present, nor carbon-dioxide production greater than the blank determinations on the apparatus. The ammonia was determined by the magnesium oxide method, and the carbon dioxide by a method previously described (9, p. 226).

THE RELATIVE NUMBERS AND DISTRIBUTION OF BACTERIA SPORES IN TWO TYPES OF SOIL AT VARIOUS DEPTHS AND SEASONS

Bacterial numbers were determined on all infusions of samples obtained for biochemical tests. These samples were obtained on August 8, November 28, January 9, April 3 and June 14, from tilled fields of Sassafras gravelly loam and of Penn shale loam. No manure or fertilizer was added to these fields during the two growing seasons during which the samples were taken. These two soils are very unlike in texture, as may be seen from the moisture record in table 1. Unheated infusions of the finer-textured shale loam gave a much higher count for the surface layers, but not for the subsoil. That a relatively greater number survived the application of heat in the surface layers of the coarser-textured gravelly loam is evident from the percentage values in table 2.

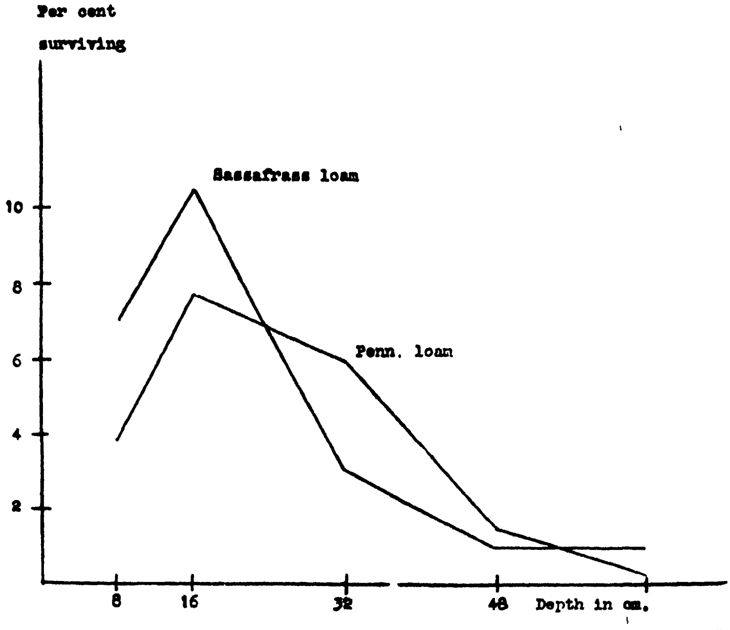

FIG. 1. CURVES SHOWING THE RELATION BETWEEN THE INCREASE IN DEPTH OF SOIL AND THE DECREASE IN BACTERIAL NUMBERS WHICH SURVIVED HEATING TO 85°C. FOR 10 MINUTES (Table 2)

In the lower layers of both types of soil, however, the numbers of spores fell off much more rapidly than the total numbers of organisms. This is evident for all of the sampling (table 2), which, when averaged, give 5.8 per cent and 8.7 per cent surviving for the first 16 cm. of surface soil, but only 2.7 per cent and 1.6 per cent for the first 32 cm. of the subsoil. This

TABLE 1

Bacterial numbers in heated and unheated infusions of Penn shale loam and of Sassafras gravelly loam

DEPTH OF SAMPLING	SASSAFRAS GRAVELLY LOAM			PENN SHALE LOAM		
	Number of bacteria		Water content of soil	Number of bacteria		Water content of soil
	Unheated	Heated		Unheated	Heated	
	Sampling of August 8					
cm.	*millions per gm.*	*millions per gm.*	*per cent*	*millions per gm.*	*millions per gm.*	*per cent*
0- 8	1 38	0 110	2 0	11 00	0 150	18.5
9-16	3 47	0 120	2 5	5 50	0 310	17.8
17-32	3 33	0 090	12 9	1 80	0 100	20.5
33-48	1 43	0 006	6 4	0 29	0 008	25 0
49-64	0 98	0 001	10.0	0 05	0.0003	20.5
	Sampling of November 28					
0- 8	5.20	0 370	16 3			
9-16	4.90	0 400	16.3			
17-32	2 00	0 053	12.3			
33-48	0 49	0 010	12 3			
	Sampling of January 9					
0- 8	6 60	0 800	16 7	29 00	2 080	31 0
9-16	4 50	1 500	16 7	25 90	4 790	31.0
17-32	1 50	0 250	9 5	3 40	0 280	26 0
33-44	0 17	0 002	9 0	3 10	0.009	30 0
	Sampling of April 3					
0- 8	7 90	0 350	13 6	6 30	0 270	25 0
9-16	4 80	0 140	23 0	8 20	0 310	25 5
17-32	1.40	0 030	11 5	1 80	9 100	25 0
33-48	0 73	0 002	10 5	0.33	0.003	24.0
49-64	0 09	0 002	11 0	0 03	None	24 5
	Sampling of June 14					
9- 8	3 96	0 162	7 5	15 50	0 186	22 0
9-16	2 31	0 117	10 2	7 00	0 245	26 6
17-32	0 61	0 037	6 4	6 10	Lost	26 6
33-48	Lost	0 009	4 2	0 22	0 006	25 0
49-64	0 21	0 001	4 2	0 25	0 005	28.2

TABLE 2

The percentage of the bacterial numbers which survived heating to 85°C. for 10 minutes

DEPTH OF SAMPLE	PENN SHALE LOAM; SAMPLING OF					SASSAFRAS GRAVELLY LOAM; SAMPLING OF					
	January 9	April 3	June 14	August 8	Average	November 28	January 9	April 3	June 14	August 8	Average
cm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0–8	7.2	4 5	1 2	1 4	3 8	6 1	12 0	4 0	4 1	8 0	6 8
9–16	18 5	3 8	3 5	4 6	7 8	8 1	33 0	3 0	5 1	3 5	10 5
17–32	8 5	5 5	Lost	5 6	6 5	2 7	1 6	2 1	6 0	2 7	3 1
33–48	0 2	1 0	0 3	2 8	1 1	2 0	1 0	0 3	Lost	0 4	0 9
49–64	None		0 2	0 6	0 21			2 0	0 5	0 1	0.9

great decrease (fig. 1) in the relative numbers of spores in the subsoil is possibly due to the fact that the subsoil flora does not undergo such extreme fluctuations in environmental conditions.

THE RELATIVE ACTIVITY OF HEATED AND UNHEATED INFUSIONS AS SHOWN BY THE AMMONIA ACCUMULATIONS

Parallel sets of unheated and heated infusions were inoculated into Erlenmeyer flasks containing 70 gm. of sterile soil and 100 mgm. of nitrogen in dried blood, the moisture content being 50 per cent of the maximum. The accumulation of ammonia nitrogen after a 7-day incubation at from 20° to 22°C. is shown in table 3. A decrease in the ammonifying power of both the heated and unheated infusions obtained from subsoil layers may be noted. The average percentage of ammonia accumulated by the heated infusions for the two types of soil at the various dates and depths of sampling was 46.6 per cent for the Sassafras gravelly loam, and 39.0 per cent for the Penn shale loam.

Thus it is evident that the spores of heated infusions became active when introduced into a favorable medium. A measure of their activity when in an associative or competitive relation to the rest of the soil flora is difficult to obtain. Working with pure cultures, Conn and Bright (3, p. 329) have presented data to show that the spore-former *Bacillus cereus* is a strong ammonifier when used alone, and that the ammonia accumulation was no greater when it was mixed with the non-spore-formers, *Pseudomonas caudatus* and *Pseudomonas fluorescens*. They also found that *B. cereus* failed to appear on plates after inoculating it into a sterile manured soil with *Ps. caudatus* and *Ps. fluorescens*.

TABLE 3

The ammonia accumulation after 7 days, resulting from inoculating sterile soi' containing dried blood with heated and unheated infusions of Penn shale loam and Sassafras gravelly loam

DEPTH OF SAMPLING	SASSAFRAS GRAVELLY LOAM			PENN SHALE LOAM		
	NH_3 — nitrogen accumulation		Relative accumulation by heated infusion	NH_3 — nitrogen accumulation		Relative accumulation by heated infusion
	Unheated	Heated		Unheated	Heated	
Sampling of August 8						
cm.	*mgm*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*
0-15	21 29	7 64	35 8	18 05	5 93	32 7
16-31	22 65	3 44	37 3	13 10	6 44	49 1
32-48	20 29	7 05	34 7	11 90	5 54	46 5
48-64	16 08	5 73	35 6	11 32	7.33	64.8
Sampling of November 28						
0 15	8 60	4 44	51 6	11 37	4 13	36 2
Sampling of January 9						
0 15	6 48	2 77	42 5	5 62	2 62	46 8
Sampling of April 3						
0-15	5 89	2 61	42 6	5 65	2 62	46 9
16-31	5 40	2 29	42 4	4 07	0 69	16 9
32-48	4 10	0 77	19 0	4 38	0 58	13 2
Sampling of June 14						
0-15	7 63	6 36	83 3	12 50	6 85	54 8
16-31	11 00	7 40	67 3	15 95	5 41	33 8
32-48	11 43	5 65	49 5	7 22	2 68	27.2
48-64	6 48	4 17	64 3	7 30	3 54	48 5
Average			46 6			39 0

THE RELATIVE ACTIVITY OF HEATED AND UNHEATED INFUSIONS AS SHOWN BY THE PRODUCTION OF CARBON DIOXIDE

Erlenmeyer flasks treated in the same way as those used for ammonia-accumulation tests were used for the carbon-dioxide work. Before using soil infusions a series of determinations were made on flasks inoculated with 5 cc. each of heated and unheated suspensions of *Bacillus subtilis*. These suspensions were obtained by adding sterile water to a large slant culture of the organism which had been incubated for 12 days. As may be seen in table 4, the production of carbon dioxide was as great for the last 3 days as for the second three of the 10-day period. The total carbon-dioxide production was about the same for the heated suspensions irrespective of the period of heating

TABLE 4

The ammonia-nitrogen accumulation and the CO_2 *production resulting from inoculating sterile soil containing dried blood with heated and unheated infusions of Bacillus subtilis*

DAYS INCUBATED	UNHEATED	INFUSIONS HEATED TO 85°C.		
		5 minutes	10 minutes	15 minutes
Carbon dioxide				
	mgm.	*mgm.*	*mgm.*	*mgm.*
0–3	26.6	14.6	20.3	8.1
4–7	42.0	29.9	33.1	35.6
8–10	35.3	28.0	28.3	31.5
0–10	103.9	72.5	81.7	75.2
Per cent of the production by unheated infusions		69.8	78.7	72.3
Ammonia-Nitrogen				
10	6.07	4.53	4.53	4.68
Per cent of the accumulation by unheated infusions . . .		74.2	74.2	76.7

TABLE 5

The production of CO_2 *resulting from inoculating sterile soil containing dried blood with heated and unheated infusions of Penn shale loam and Sassafras gravelly loam*

DEPTH OF SAMPLING	DAYS INCUBATED	PENN SHALE LOAM			SASSAFRAS GRAVELLY LOAM		
		Unheated	Heated	Relative accumulation by heated infusions	Unheated	Heated	Relative accumulation by heated infusions
Sampling of August 8							
cm.		*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*
0–16	0–4	90.1	56.3		90.1	66.3	
	5–7	178.7	167.8		201.5	143.3	
	0–7	268.8	224.1	83.3	291.6	209.6	71.9
17–32	0.4	53.0	59.0		104.8	58.8	
	5–7	207.2	165.4		204.3	175.0	
	0–7	260.2	224.4	86.2	309.1	233.8	75.6
Sampling of June 14							
0–16	0–4	64.4	46.4		72.3	59.8	
	5–7	75.0	57.4		54.6	56.1	
	0–7	139.4	103.8	74.4	126.9	115.9	90.5
17–32	0–4	72.0	40.8		77.4	53.1	
	5–7	74.6	53.3		62.7	40.3	
	0–7	146.6	94.1	64.2	140.1	93.4	66.7
Average relative accumulation				77.0			76.2

TABLE 6

Triplicate determinations of bacterial numbers given in table 1

DEPTH OF SAMPLING	SASSAFRAS LOAM						PENN LOAM					
	Unheated inoculum			Heated inoculum			Unheated inoculum			Heated inoculum		
	1	2	3	1	2	3	1	2	3	1	2	3
	Sampling of August 8											
cm.												
0- 8	1 43	1 33		0 12	0 11	0 11	11 0	12 2	9 8	0 19	0 17	0 10
9-16	3 87	2 37	3 17	0 16	0 09	0 10	4 9	7 3	4 9	0 38	0 26	0 28
17-32	3 18	3 63	3 18	0 09	0 08		1 6	1 5	2 3	0 10	0 11	0 10
33-48	1 39	1 50	1 60	0 007	0 005	0 005	0 26	0 29	0 33	0 007	0 011	0 007
49-64	1 08	0 94	0 91	0 001	0 001	0 002	0 06	0 05	0 04	0 0003	0 003	0 0002
	Sampling of November 28											
0- 8	5 4	4 5	5 6	0 418	0 311	0 382						
9-16	4 9			0 441	0 370	0 323						
17-32	2 3	1 60		0 051	0 051	0 057						
33-48	0 34	0 45		0 010	0 011	0 009						
	Sampling of January 9											
0- 8	6 14	5 50	7 20	0 90	0 69		29 0			1 74	2 17	2 32
9 16	4 9	4 7	4 0	1 44	1 68		24 3	27 5		3 77	5 80	
17-32	1 1	1 8	1 8	0 30	0 25	0 20	3 4	3 3	3 6	0 35	0 22	
33-48	0 20	0 20	0 12	0 002	0 002	0 001	0 32	0 30		0 007	0 007	
	Sampling of April 3											
0- 8	7 7	8 2		0 32	0 33	0 39	5 8	6 1	6 9	0 25	0 23	0 32
9 16	6 2	3 5		0 17	0 11	0 13	8 0	6 5	10 0	0 29	0 33	0 32
17 32	1 4	1 6	1 4	0 038	0 033	0 030	1 4	2 2	1 7	0 90	0 10	0 10
33-48	0 81	0 64	0 75	0 002	None	None	0 45	0 27	0 27	0 003	0 003	
49-64	0 09	0 09	None	0 002	0 002	None	None	0 03	None	None	None	None
	Sampling of June 14											
0- 8	3 76	3 68	4 43	0 158	0 145	0 182	12 90	16 2	17 3	0 159	0 171	0 186
9-16	2 24	2 45	2 24	0 078	0 122	0 111	7 76	6 28		0 163	0 231	0 245
17-32	0 59	0 54	0 69	0 032	0 032	0 048	5 90	6 25				
33-48				0 008	0 009	None	0 20	0 20	0 25	0 007	0 008	0 006
49-64	0 175	0 219	0 228	0 001	0 0004	None	0 26	0 25	0 23	0 0005	0 0004	0 0005

TABLE 7

Duplicate determinations of ammonia accumulations given in table 3

DEPTH OF SAMPLING	SASSAFRAS LOAM				PENN LOAM			
	Unheated infusions		Heated infusions		Unheated infusions		Heated infusions	
	1	2	1	2	1	2	1	2
Sampling of August 8								
cm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0–15	21 88	20 70	8 02	7 25	18 8	17 30	7 40	4 46
16–32	21 72	23 50	8 30	9 17	13 85	12 35	8 15	4 72
33–48	20 98	19 60	7 73	6 36	12 46	11 33	5 86	5 22
49–64	16 40	15 76	5 60	5 86	10 05	12 60	7 77	6 88
Sampling of November 28								
0–15	9 6	8 11	3 70	5 17	11 19	11 56	4 43	3 82
Sampling of January 9								
0–15	6 32	6 64	3 20	2 34	5 29	6 15	2 34	2 91
Sampling of April 3								
0–15	5 72	5 97	2 80	2 42	5 59	5 70	2 32	2 92
16–31	5 46	5 34	2 16	2 42	3 81	4 32	0 76	0 51
32–48	3 74	4 46	0 64	0 89	4 70	4 06	0 51	0 64
Sampling of June 14								
0–15	7 95	7 30	6 61	6 10	13 30	11 70	7 50	6 20
16–31	11 50	10 39	7 50	7 30	12 90	19 00	5 54	5 28
32–48	11 75	11 11	5 40	5 90	7 50	6 94	1 21	4 14
49–64	5 91	7 05	4 00	4 34	8 70	5 90	2 50	4 64

TABLE 8

Duplicate determinations of ammonia and of carbon dioxide given in table 4

DAYS INCUBATED	UNHEATED INFUSIONS		HEATED FOR 5 MINUTES		HEATED FOR 10 MINUTES		HEATED FOR 15 MINUTES	
	1	2	1	2	1	2	1	2
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0– 3	26 6	26 6	14 7	14 4	20 3	31 2	3 2	13 0
4– 7	39 0	45 0	29 9	29 9	33 1	47 1	34 5	36 6
8–10	36 7	33 9	25 2	30 8	28 1	Lost	27 7	35 3
0–10	102 3	105 5	68 8	75 1	81 7		65 4	84 9
Ammonia-nitrogen for 10 days in flasks used for the above CO_2 determinations								
0–10	6 17	5 97	4 67	4 38	4 41	4 65	4 98	4 38

TABLE 9

Duplicate determinations of CO_2 production given in table 5

DEPTH OF SAMPLING	DAYS INCUBATED	SASSAFRAS LOAM				PENN LOAM			
		Unheated infusions		Heated infusions		Unheated infusions		Heated infusions	
		1	2	1	2	1	2	1	2
		Sampling of August 8							
cm.		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0-16	0-4	92.9	87.2	69.4	63.2	72.9	107.4	60.0	52.7
	5-7	203.0	200.0	143.3	Lost	165.3	192.0	164.5	171.0
	0-7	295.9	287.2	212.7		238.2	299.4	224.5	273.7
17-32	0-4	104.8		58.8		53.0		59.0	
	5-7	204.3		175.0		207.2		165.4	
	0-7	309.1		233.0		260.2		224.9	
		Sampling of June 14							
0-16	0-4	69.0	75.6	58.8	60.8	63.3	65.4	48.0	44.7
	5-7	58.2	51.0	49.4	62.7	75.3	74.6	69.0	45.7
	0-7	127.2	126.6	108.2	123.5	138.6	140.0	117.0	90.4
17-32	0-4	77.4		53.1		72.0		40.8	
	5-7	62.7		40.3		74.6		53.3	
	0-7	140.1		93.4		146.6		94.1	

between the limits of 5 and 15 minutes. This lack of effect of the period of heating was also observed in preliminary work on the numbers surviving in soil infusions. The average carbon-dioxide production by this heated suspension of *B. subtilis* was 73.6 per cent of the production by the unheated for a 10-day period.

The ammonia accumulation in the flasks from which the carbon dioxide was determined is also given in the table, and averaged 75 per cent for the heated infusions. This close correlation with the relative carbon-dioxide production has been previously shown for bacterial cultures (9, p. 242).

Table 5 gives the production of carbon dioxide from infusions of the two types of soil for the sampling of June 14 and August 8. The average per cent of carbon dioxide by the heated infusions of Penn shale loam for a 7-day period was 77 per cent of the production by the unheated infusions. The corresponding value for the Sassafras gravelly loam was 76.2 per cent.

It is believed that the higher production for the samples of August 8 was due to a higher temperature of incubation, as the refrigeration apparatus was not working properly during the month of August, and the incubation temperature was higher than from 20° to 22°C.

CONCLUSIONS

1. Infusions from the five successive layers of the upper 64 cm. of soil were heated to 85°C. for 10 minutes, and the carbon-dioxide production, ammonia accumulation and numbers were compared with a similar treatment with unheated infusions.

2. In the surface soil of the finer-textured loam, 5.8 per cent, and of the coarser loam, 8.7 per cent, of the original count survived a temperature of 85°C. for 10 minutes.

3. The relative number surviving in the subsoil was much less, being 2.7 and 1.6 per cent, respectively.

4. Very few fungus colonies, and no actinomycetes or chromogenic types were observed in the plates inoculated with heated infusions.

5. For a 7-day period the average ammonia accumulation resulting from inoculating with heated infusions was 46.6 per cent of that with unheated infusions obtained from Sassafras gravelly loam and 39 per cent when infusions from Penn shale loam were used.

6. For a 10-day period the carbon dioxide produced by inoculating with heated infusions of *B. subtilis* was about the same whether the infusion had been heated for 5, 10, or 15 minutes, and averaged 73.6 per cent of the amount produced by unheated infusions.

7. For a 7-day period the average carbon-dioxide production resulting from the use of heated infusions of Penn shale loam was 77 per cent of that obtained with unheated infusions. The corresponding value for Sassafras gravelly loam was 76.2 per cent.

8. The accumulation of NH_3 and the production of carbon dioxide resulting from inoculating a sterile soil with heated infusions correlate with each other in showing that the bacterial spores of the soil are capable of energetic activity when supplied with sufficient food and moisture. As to whether they would be equally active in liberating plant-food when competing with the actinomycetes, non-sporulating bacteria, and fungi in a normal soil flora, is a subject which needs further investigation. When sufficient information concerning the importance and environmental requirements of the various groups of soil microörganisms has been obtained, the way will be ready for a more rational control of soil conditions to enhance the action of the beneficial groups and to check those which are detrimental.

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STUDIES ON THE REACTION OF PLANT JUICES[1]

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Comparatively little investigation has been done on the actual reaction[3] of plant juices upon plant metabolism, notwithstanding its importance. Loeb, in 1919, in a series of five papers on amphoteric colloids in the Journal of General Physiology, has shown the importance of the reaction upon the retention and excretion of ions from protein-containing solutions, as well as upon other physical and chemical properties of amphoteric colloids. The factors that may affect the reaction of plant juices are numerous, and as yet are but little understood.

Inasmuch as the soil reaction is one of the important factors affecting the growth of plants, it is quite possible that the degree of acidity of plant juices may be correlated with the reaction of the soil upon which the plants are grown. It has already been shown by Fred and Davenport (4) that the reaction of the medium markedly affects the metabolism of legume bacteria, and that they are rendered inactive at a more or less well-defined critical pH value of the medium. In addition to the reaction of the substrate, meterological and hereditary factors may be considered as possibly affecting the reaction of plant juices.

The present paper deals with the actual and the total acidities and the total alkalinity of the juices of a number of plants of agricultural importance, together with a study of the influence of liming the soil upon these acidities. The actual acidities of the different parts of plants and their relation to one another are discussed. The effects of age, lack of chlorophyll and changes in illumination, upon the actual and total acidities of plant juices also are considered.

The actual acidity of soil solutions (27) from which plants derive food has been the subject of considerable investigation (25). The studies of Hoagland (12) on the relation of the nutrient solution to the composition and reaction of the cell sap of barley plants, is a step in the right direction.

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[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] The writer has been obliged to conclude his present studies and finds it advisable to present the data thus far obtained.

[3] By actual acidity is meant the amount of dissociated acid. The total acidity includes both the dissociated and undissociated acids.

It has been shown that the actual reaction of the juice of most plants is markedly acid (7, 8), and that only occasionally are species found with cells the juices of which are neutral or slightly alkaline in reaction (5). However, Kappen (13) has recently reported a slight alkalinity in the juice of wheat roots. Rose (20) has also reported the interesting observation that as germination begins, the reaction of the embryos of *Sambucus* changes from alkaline to acid but that the endosperm remains alkaline. The juice of succulent plants has been found by Hempel (11) to be quite acid in reaction, and it was she who first elucidated the nature of the buffer processes existing in the juice of such plants.

For previous work upon reaction as regards both plants and animals, the bibliography compiled by Schmidt and Hoagland (21) is very complete.

METHOD OF DETERMINING THE ACIDITY OF PLANT JUICES

The determination of both the actual and the total acidity (10) of plant juices was carried on by the use of the hydrogen-electrode. It was found that the juice obtained from the plants growing in a single pot was frequently insufficient for carrying on a determination of the actual reaction when a 3-by-10-cm. glass titration tube was used. An effort was made to use the indicator paper method (9) but it was found quite impossible to secure clear or nearly clear plant juice even after filtration by various methods and repeated centrifuging. Unless the juice was very acid, it retained a deep green color. Moreover, it was considered inadvisable to use two methods (the indicator paper method is less accurate than the electrometric) in making comparative determinations, especially in case differences are small.

Accordingly a small hydrogen-electrode vessel was devised as shown in figure 1, in order that determinations of the actual acidity could be made when only three or four drops of plant juice were available. The essential point in the construction of the apparatus is to make the bulb F of such size and shape that a few drops of solution suffice to cover the lower end of the glass tube for the KCl contact and gas-electrode. The small hydrogen-electrode vessel was found to be more convenient than the larger vessel except when the juice was to be titrated, in which case the larger vessel was used. A small quantity of plant juice was found to be preferable to a larger quantity, for with small amounts of juice equilibrium was more rapidly attained. The results obtained with the small apparatus were compared with the results obtained with the larger hydrogen-electrode vessel.

The juice from fresh medium red clover grown out-of-doors was obtained by first crushing the cells in a tinned meat grinder and then pressing out the juice through a clean linen cloth. The extracted juice was not centrifuged. Four drops of the plant juice (0.12 cc.) were introduced with a pipette into the small hydrogen-electrode vessel. The actual reaction of the plant juice was found to be pH = 6.02. The original juice was left standing ½ hour and a second quantity amounting to 4 drops (0.13 cc.) was used. The actual reaction was found to be pH = 6.06. A 7-cc. sample of the original juice was then run with the larger hydrogen-electrode vessel and the actual reaction of the juice was found to be pH = 6.06. In the determination of the actual reaction of some very minute quantities of plant juice, this small type of hydrogen-electrode apparatus served a very useful purpose and facilitated considerably the numerous determinations of the reaction made upon agricultural plants.

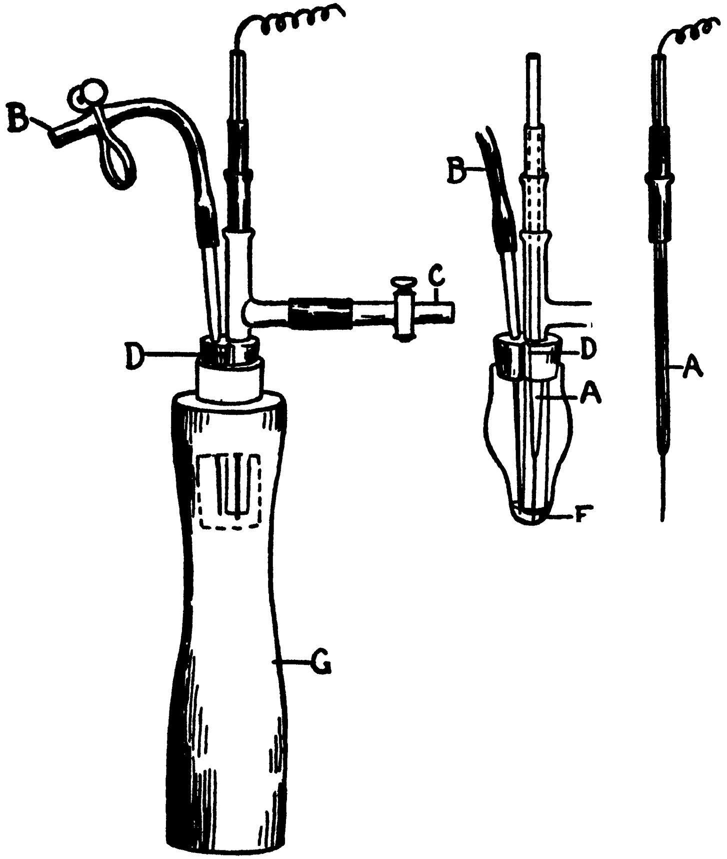

FIG. 1. THE SMALL HYDROGEN ELECTRODE APPARATUS DRAWN TO SCALE

A, Hydrogen-electrode wire at lower end connected with mercury to wire connection at upper end. *B*, Connection with KCl to the calomel electrode. *C*, Hydrogen inlet *D*, Holes for outlet excess gas. *F*, Plant juice. *G*, Rubber holder for hydrogen electrode vessel with glass tube inserted at lower end for rigidity, and window at upper end for observation.

Besides determining the actual reaction of plant juice, it was found possible, whenever 5 cc. or more of juice was available, to titrate the total acid by means of the hydrogen-electrode. It is possible to titrate much smaller quantities of juice, but it is not convenient. By titrating the total acidity of the plant juice it is possible to obtain an idea of its buffer action.

The following titrations, taken at random from a large number of determinations, serve to indicate the degree of accuracy that can be secured by the electrometric titration (10) of aliquots of the same sample of expressed plant juice when the readings of E. M. F. are made with a voltmeter. The results are given in table 1.

TABLE 1

Agreement of duplicate determinations of actual acidity upon adding 0.0689 N sodium hydroxide to 10 cc. of juice of medium red clover tops

FIRST TRIAL		SECOND TRIAL	
Actual acidity	NaOH added	Actual acidity	NaOH added
pH	*cc.*	pH	*cc.*
6.08	0	6.10	0
6.92	1.0	6.88	1.0
7.53	2.0	7.53	2.0
8.09	3.0	8.07	3.0
8.41	4.0	8.41	4.0
8.69	5.0	8.68	5.0
9.00	6.0	9.02	6.0
9.34	7.0	9.34	7.0
9.63	8.0	9.60	8.0

Without plotting the values, it is obvious that the two successive titrations check very closely. In an electrometric titration, it is frequently possible to observe errors in the reading of the burette or the E. M. F. by the fact that such points ordinarily do not fall close to the trend of the titration curve.

EFFECT OF STANDING ON THE ACTUAL AND TOTAL ACIDITIES

The previous treatment of the plants and the time elapsing between expressing and using the plant juice may influence the actual and total acidities obtained. The total acidity of plant juice will differ according to the pH we select as our end-point. If we select pH = 6.81 we have the turning point for litmus; if we select pH = 8.31 we have the turning point for phenolphthalein. Hence in titrating for total acidity we can tell at once from the curve the amount of alkali required for titration to any given pH and consequently to the color change for any indicator. For example, some medium red clover plants 12 to 14 inches high, growing on a limed plot (pH = 7.53) were cut off just above the surface of the soil at 11.30 a.m. and

TABLE 2

Effect of standing on actual and total acidities of medium red clover juice
10 cc. of juice titrated with 0.0684 *N* sodium hydroxide

ACTUAL ACIDITY	PLANTS FROM LIMED SOIL	ACTUAL ACIDITY	PLANTS FROM UNLIMED SOIL
I (1.50 p.m.)		I (8.15 p.m.)	
pH	*cc.* NaOH *added*	pH	*cc.* NaOH *added*
6.12	0.0	5.94	0.0
7.31	1.0	6.45	1.0
8.05	2.0	6.94	2.0
8.56	3.0	7.53	3.0
8.90	4.0	8.07	4.0
9.20	5.0	8.39	5.0
9.46	6.0	8.75	6.0
II (3.05 p.m.)		II (9.30 p.m.)	
6.12	0.0	5.82	0.0
6.84	1.0	6.45	1.0
7.87	2.0	6.94	2.0
8.22	3.0	7.33	3.0
8.41	4.0	7.97	4.0
8.98	5.0	8.22	5.0
9.35	6.0	8.66	6.0
III (3.55 p.m.)			
6.03	0.0		
6.84	1.0		
7.56	2.0		
8.00	3.0		
8.36	4.0		
8.68	5.0		
9.05	6.0		
IV (5.00 p.m.)			
6.04	0.0		
6.72	1.0		
7.51	2.0		
7.88	3.0		
8.34	4.0		
8.69	5.0		
9.08	6.0		
V (7.10 p.m.) Freshly expressed and centrifuged			
6.10	0.0		
6.72	1.0		
7.39	2.0		
7.97	3.0		
8.69	5.0		
9.02	6.0		

placed in beakers containing water. Some medium red clover plants 8 to 10 inches high from an unlimed but otherwise similarly fertilized plot (pH = 6.19) were treated in the same way. Just prior to the first determination in each of the two series of table 2, the lower portion of the plants that had been in the beakers of water were cut off and discarded, and the juice of the upper remaining portion was expressed and centrifuged 15 minutes. The juice of the plants from the limed soil was kept in the dark and 10-cc. aliquots were titrated at intervals throughout the afternoon. The juice of the plants from the unlimed soil was not expressed until in the evening and only two successive titrations were made.

From the table it is evident that on standing in the dark there is a tendency for the medium red clover juice to undergo changes in its actual and total acidities. In the first four determinations on equal aliquots of the same original juice of plants grown on the limed soil there was an increasing total acidity, whereas the fifth determination, made upon freshly expressed and centrifuged juice, behaved more like determinations III and IV than like I and II as regards total acidity and more like determinations I and II than like III and IV as regards initial actual acidity. The juice of the unlimed clover had a greater actual and total acidity, upon allowing the expressed and centrifuged juice to stand in the dark, than when used immediately. When the juice of the limed clover plants was left standing over night for 24 hours and its actual acidity again determined, it was found that the value for the actual acidity had changed from pH = 6.12 to pH = 5.91. This marked increase in actual reaction upon oxidation of the juice is in accord with the observations of Miss Hempel (11) for the juice of succulent plants. The results in table 2 cannot be compared with those in table 1 as it is essential to use fresh plants. Furthermore, as will be indicated, the soil solution and other factors may affect the actual and total acidities of plants.

EFFECT OF LIMING ON THE ACTUAL AND TOTAL ACIDITIES

On comparing determination I of the unlimed clover, in table 2, with determinations I and V of the limed clover, it is found that the unlimed clover has a greater actual and a greater total acidity than the limed clover, regardless of the pH which might be chosen as the end-point.[4]

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[4] In order to compare determination I of the unlimed with determination V of the limed clover as regards total acid, we select any pH as our turning point and note the amount of alkali required to bring the juice to the selected pH end-point. Thus for example, in determination I of the unlimed clover we find that 5 cc. of alkali are required in order to bring the pH of the juice to 8.39, whereas in determination V of the limed clover less than 5 cc. of the alkali is required in order to bring the pH of the juice to 8.39, since 5 cc. carries us 0.30 beyond the end-point pH = 8.39. This shows that the juice of the unlimed clover has a greater total acidity than the juice of the limed clover with reference to pH = 8.39 as our end-point.

It seems important, therefore, to consider the effect of liming the soil on the actual and total acidities of plants grown on such soils. Various crops were grown on Plainfield sand, using pots containing 11,500 gm. of soil. Plant tissue was available from these pots through cooperation with Dr. C. B. Clevenger.

Half of the pots received sufficient ground limestone to neutralize all of the acid as indicated by the Truog test (24), and all the pots received 2 gm. of dipotassium phosphate. The seeds were planted March 1.

The actual and the total acidities of 6 cc. of alsike clover juice were determined for both the limed and the unlimed Plainfield sand. The tops were cut May 13 and the juice was centrifuged for 10 minutes after being expressed. In none of the experiments in this paper was there any water added to the crushed plant tissue or the expressed juice. The values obtained for alsike clover are given in the form of curves in figure 2.

From the curves, it is evident that the actual as well as the total acidities of the juice of the tops of alsike clover plants grown without liming are greater in every case than those with liming. If, for example pH = 8.0 is chosen as the end-point, it is seen at once that the 6-cc. sample of juice from the plants with liming required 2 cc. of alkali, whereas that from plants without liming required 3 cc. to bring the juice to pH = 8.0.

The 7-cc. samples of juice of serradella tops cut on June 2 from limed and unlimed Plainfield sand gave a different relationship (figure 2) from that described for alsike clover. The actual acidity of the plants with liming was greater than that of the plants without liming. As alkali was added, at first the total acidity of the juice of the limed plants is greater, then less, and then greater than that of the juice of the unlimed plants.

When the 8-cc. samples of juice of the tops of garden bean plants, grown on limed and on unlimed Plainfield sand and cut on May 13, were examined, the results represented by the curves in figure 2 were obtained. It is found that the actual as well as the total acidities of the juice of plants with liming was greater than that of the plants without liming. These results might be explained possibly by such a fact as Gile and Ageton (6) have found for beans, namely, that in the ash of bush beans the amount of lime was slightly decreased rather than increased with increasing amounts of calcium carbonate in the soil.

Barley plants, not yet in bloom, were cut off at the surface of the limed and unlimed Plainfield sand on May 12. The expressed plant juices were centrifuged for 15 minutes. The results obtained for the actual and the total acidities of 15-cc. samples of the juices are plotted in figure 3. The initial actual acidity was greater in the plants from unlimed than from limed soil. The total acidity in both cases was practically the same for any given pH as the end-point.

When 15-cc. samples of juice obtained from oat plants cut May 9 on limed and unlimed Plainfield sand were examined, it was found (fig. 3) that the initial actual acidities of the juices were practically the same. However, as regards total acidity, it was found that no matter which pH was chosen as the end-point, the plants with liming showed a greater total acidity than those without liming.

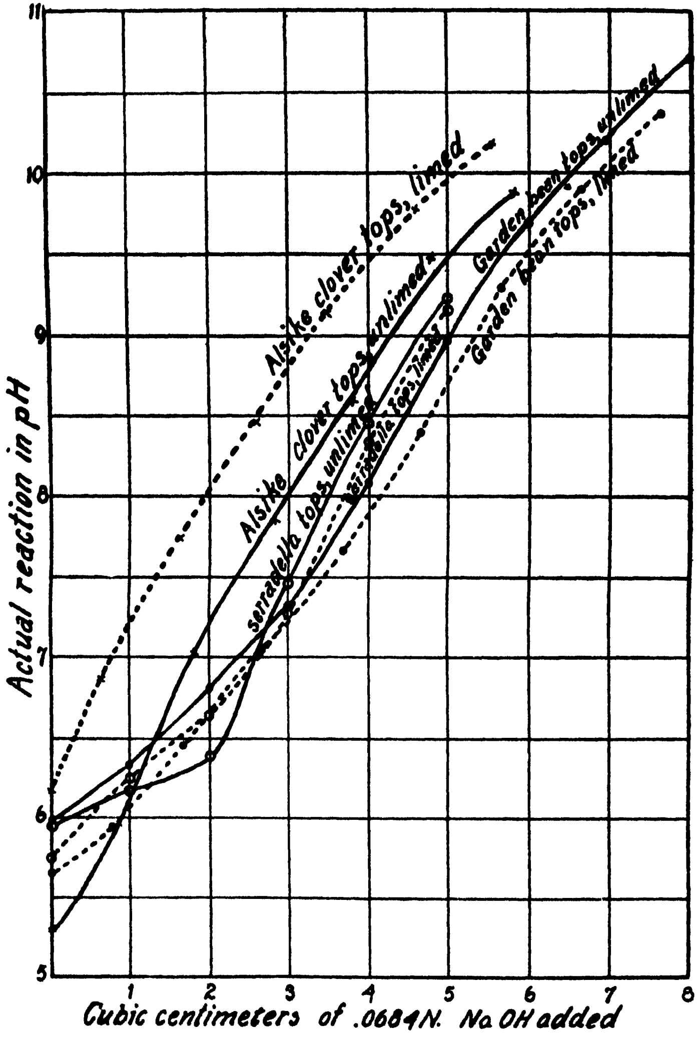

FIG. 2. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES (EXPRESSED IN TERMS OF CUBIC CENTIMETERS OF 0.0684 *N* SODIUM HYDROXIDE) OF THE JUICES OF PLANTS GROWN ON LIMED AND UNLIMED SOIL

Corn plants growing on soils similar to those of the oat plants were cut May 9. The titration curves of 15-cc. samples of both juices resemble those for oat plants. The initial actual acidities with and without liming were the same. However, for any chosen end-point (pH) the total acidity of the juice of the corn with liming was greater than that without liming.

Truog (25) has suggested that one of the chief functions of calcium, when taken up by the plant in the carbonate or bicarbonate form, is to neutralize and precipitate acids within the plant and thus prevent an injurious degree of acidity. How these irregular results may be harmonized with this suggestion will now be discussed.

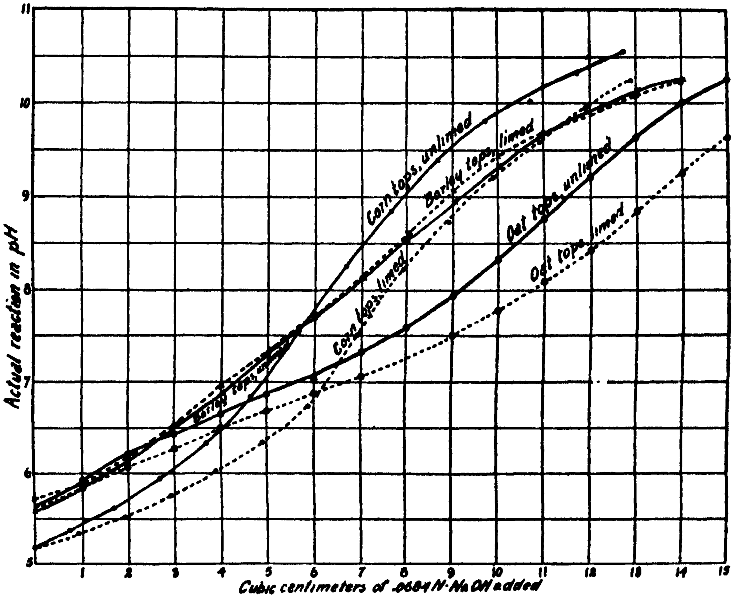

FIG. 3. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF THE JUICE OF BARLEY, OAT, AND CORN PLANTS GROWN ON LIMED AND UNLIMED SOIL

Some of the results from plants grown upon Plainfield sand are most irregular if not abnormal when compared with those from plants grown upon other types of soil. Frequently the addition of lime to Plainfield sand had an unfavorable effect on plant growth even with plants that ordinarily are benefited by liming. This soil thus gave some unexpected results.

In the experiments just described there was not taken into consideration the possibility that differences in actual and in total acidities may exist in different parts of the same plant. With succulent plants in which the leaves are of large size it is relatively a simple matter to separate the leaf, stem, and root portions of the plants and to compare the actual and the total acidities of corresponding parts. With alfalfa, clover and other plants of a similar nature, it requires considerable time and patience to separate the leaves, stems, and roots from one another in order to obtain sufficient of the plant juice with which to carry on the determinations.

In some of the determinations of the actual acidity of the plant juice of rhubarb leaf-stalk, it was found (8) that the actual reaction varied considerably according to the part of the plant from which the juice was expressed. The observations of Rose (20) upon the reaction of different parts of seed tend to emphasize the fact that it is essential to compare the expressed juices of corresponding parts of plants.

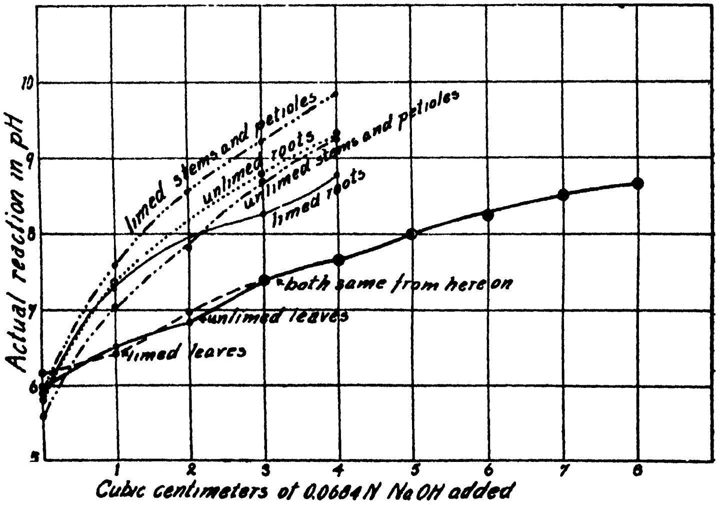

FIG. 4. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF LEAVES, STEMS, PETIOLES, AND ROOTS OF MEDIUM RED CLOVER PLANTS GROWN WITH AND WITHOUT LIMING

The results reported in this paper (fig. 4) point out the fact that leaves, stems, and roots may differ quite considerably in their actual reaction.

In order to control the conditions as much as possible, fresh medium red clover was secured from the adjoining farm plots; one plot had received lime, the other had not. The actual reaction of the limed plot (aqueous extract) was pH = 7.53 and that of the unlimed plot pH = 6.19. The plants from the limed plot were 20 to 24 inches high, whereas those from the unlimed plot were 10 to 18 inches high. When the plants were cut on May 30 those from the unlimed plot appeared a lighter green than the others. In the first determination the leaflets were picked from each plant and their juice expressed in the usual manner. In order to observe errors that might be introduced as a result of changes in the juice during a 20-minute period of centrifuging, a determination of the actual acidity of a portion of the juice was made immediately after the juice was expressed, whereas the remainder of the juice was centrifuged. The results obtained for the effect of centrifuging on the initial actual acidities of the juices may be stated briefly as follows:

Uncentrifuged

Limed plants	
Leaves	pH = 6 19
Stems and petioles	pH = 5 95
Unlimed plants	
Leaves ..	pH = 6 02
Stems and petioles	pH = 5 63

Centrifuged

Limed plants	
Leaves	pH = 6 12
Stems and petioles	pH = 5 89
Unlimed plants	
Leaves	pH = 5 99
Stems and petioles	pH = 5 62

Obviously, only a slight increase in actual reaction of the juice of the above-ground parts occurred during the centrifuging.

The curves (fig. 4) represent the actual and the total acidities (expressed in terms of 0.0684 *N* sodium hydroxide) of the 7-cc. samples of different parts of medium red clover plants. The titration curves make it evident that the initial actual reaction of the leaves, stems and petioles, and roots of plants from limed soil was less than that of the corresponding parts of the plants from unlimed soil. The total acidities of the leaf juices were quite similar in both cases. The juice of the stems and petioles of the plants from limed soil had a smaller total acidity than that of the plants from unlimed soil. The total acidity of the roots without liming was at first greater than that with liming, but became smaller than that with liming as the titration proceeded.

However, the day previous to the above experiment, two lots of medium red clover roots were secured from the same plot as before and determinations made of the actual reactions of the uncentrifuged juice. From table 3 it is evident that the roots with liming had a slightly greater actual acidity than the roots without liming and that the results obtained for the root juice (fig. 4) are reversed.

Timothy and winter wheat plants were cut June 11 at the surface of the soil of the same limed and unlimed plots in which the medium red clover was growing. The actual reactions recorded in table 3 indicate that the effect of liming the soil was to decrease the actual acidity of the juice of timothy and wheat.

White mustard plants were grown on the limed and the unlimed Plainfield sand from March 1 to June 13. When the tops were cut off they were very unequal in size on the limed and unlimed pots. The roots from the limed and unlimed soil with about an inch of the attached stem were the only parts utilized in the determinations of the actual acidities of the juices.

Table 3 makes it clear that the juice of the roots with liming showed a less actual acidity than without liming. Whenever roots were used they were repeatedly washed with pure distilled water and then shaken free of any adhering moisture, after which they were further dried by drawing them over a good grade of filter paper. These determinations were made possible by using the small hydrogen-electrode vessel. The tops were so unequal in size (plate 1) that it seemed best to utilize the tops of white mustard at an earlier stage, when they were more nearly equal in size and a more fair comparison of the actual acidities of the juices would be possible.

TABLE 3

Actual acidities of juice of different parts of plants grown on limed and unlimed soil

PLANT	PART OF PLANT	TREATMENT	LIMED SOIL	UNLIMED SOIL
			pH	pH
Medium red clover, lot 1	Roots	Uncentrifuged	5.87	5.91
Medium red clover, lot 2	Roots	Uncentrifuged	5.82	5.88
Timothy	Leaves, stems, young spikes	Uncentrifuged	6.17	6.14
Timothy	Leaves, stems, young spikes	Uncentrifuged	6.19	6.12
Winter wheat, lot 1	Tops (pollen stage)	Centrifuged 10 minutes	6.33	5.95
Winter wheat, lot 2	Leaves, stems, no spikes	Centrifuged 10 minutes	6.12	5.77
White mustard	Roots with lower 1 inch of stem	Uncentrifuged	5.91	5.62
White mustard	Entire plants	Uncentrifuged	5.78	5.48
Corn	Tops	Centrifuged	5.48	5.48
Field peas	Tops	Uncentrifuged	6.53	6.80
Buckwheat	Entire plants	Uncentrifuged	5.97	5.48
Alfalfa	Tops	Centrifuged	6.19	5.99
Baltic alfalfa no. 550	Roots	Uncentrifuged	6.12	6.71
Common South Dakota alfalfa, no. 363	Roots	Uncentrifuged	6.12	6.06
Alsike clover	Roots	Uncentrifuged	5.8[illegible]	5.68

White mustard seed was sown in Colby silt loam in pots containing 11,500 gm. of the soil. To some pots no lime was added; to others 1 per cent sufficient ground limestone was added, to neutralize the acidity as shown by Truog's method. Each pot received 25 cc. of water containing 1 gm. of dipotassium phosphate.[4] The other plants grown on Colby silt loam, which are to be mentioned later, received the same soil conditions as white mustard.

The juice of the white mustard plants was so small in amount that in order to obtain any at all, it was necessary to place the entire plants inside a linen cloth and after crushing the plant cells in a porcelain mortar, the juice was expressed by wringing the cloth. The actual acidities of the juices of the plants under both treatments, as regards liming, are given in table 3. Even at this early stage in the growth of the plants, a distinct decrease in the actual reaction of the juice of plants with liming as compared with that of plants without liming is evident.

When corn plants about 10 to 12 inches high that were grown on limed and on unlimed Colby silt loam were used for determinations of actual acidities, the juice in both cases gave a value of pH = 5.48. The small hydrogen-electrode vessel was not available at the time and separate determinations on the leaves and stalks were not made.

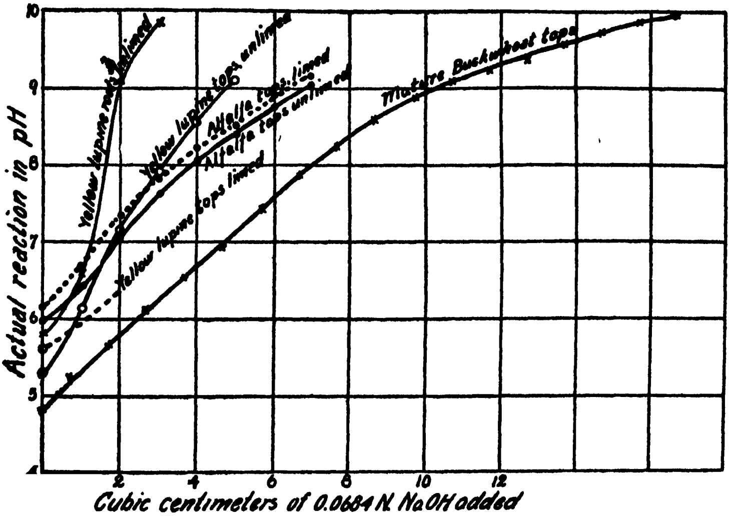

FIG. 5. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF THE JUICE OF BUCKWHEAT TOPS AND OF THE JUICES OF ALFALFA TOPS, LUPINE TOPS, AND LUPINE ROOTS GROWN WITH AND WITHOUT LIMING

The actual acidities of the juices of field pea tops and of entire buckwheat plants grown on limed and unlimed Colby silt loam were determined. The field pea plants in each case were about 8 inches high; the buckwheat plants in each case were about 5 to 6 inches high. The results are given in table 3.

In table 3 it is seen that the acidity of the juice of the pea plants with liming was greater than that of the plants without liming. With buckwheat the converse was true, and the actual reaction with liming was much less than without liming.

Plants from limed and unlimed plots at Monroe, Wisconsin, were brought into the laboratory shortly after being dug up. The roots were well covered with soil and the tops were in excellent condition. Table 3 gives the results for alfalfa tops and several lots of roots, obtained from limed and unlimed plots. Figure 5 gives the titration curves of 9.5-cc. samples of juice of the alfalfa tops from limed and unlimed soil and shows that liming decreased both the actual and total acidities.

Viewing table 3 as a whole, it is found that in one case, liming the soil was followed by no change in the actual acidity of the plant juice. However, in 10 out of 14 cases the addition of lime was followed by a decrease in the actual acidities of the plant juices.

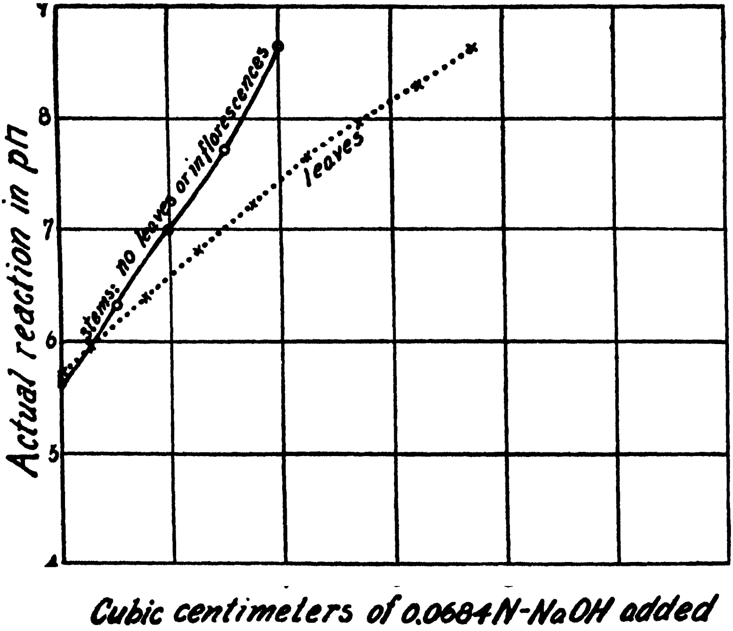

FIG. 6. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF THE JUICE OF THE LEAVES AND STEMS OF ORCHARD GRASS

Although yellow lupines do not grow well on limed Plainfield sand, it was possible to secure sufficient of the plant juice to obtain an idea concerning the effect of liming on the actual acidity of the juice. The plants had grown from March 1 to June 3. The plants without liming were in bloom, whereas the plant with liming was not in bloom and was only about one-half as high as the former. Unfortunately, insufficient juice was obtained from the single plant grown on limed soil to carry on a titration for the total acidity.

In both cases the buds, flowers, and nodules were discarded. A 7-cc. sample of juice was used in each titration. The results obtained for yellow lupine are given in the form of curves in figure 6.

The curves indicate that lime decreased the actual acidity of the juice of yellow lupine tops. Without liming the total acidity of the juice of the tops was greater than that of the roots.

TABLE 4

A summary of the effect of liming the soil upon the actual and total acidities of plant juices

PLANT	ACTUAL ACIDITY	ACTUAL ACIDITY OF JUICE		TOTAL ACIDITY
		Limed	Unlimed	
		pH	pH	
Alfalfa tops	Decrease	6.19	5.99	Decrease
Alfalfa roots (Baltic no. 550)	Increase	6.12	6.21	
Alfalfa roots (Common South Dakota no. 363)	Decrease	6.12	6.06	
Alsike clover roots	Decrease	5.84	5.68	
Alsike clover tops	Decrease	6.19	5.28	Decrease
Barley tops	Decrease	5.72	5.62	No difference
Buckwheat (entire seedlings)	Decrease	5.97	5.48	
Corn tops	No difference	5.19	5.19	Slight increase
Corn tops	No difference	5.48	5.48	
Field peas tops	Increase	6.53	6.80	
Garden bean tops	Increase	5.65	5.97	Increase
Lupine yellow tops	Decrease	5.63	5.31	
Lupine, yellow roots			5.80	
Medium red clover roots	Increase	5.87	5.91	
		5.82	5.88	
Medium red clover tops	Decrease	6.12	5.94	Decrease
Medium red clover leaves	Decrease	6.19	6.02	No difference
Medium red clover stems and petioles	Decrease	5.95	5.63	Decrease
Medium red clover roots	Decrease	5.92	5.84	Decrease then increase
Mustard, white, roots	Decrease	5.91	5.62	
Mustard, white entire plants	Decrease	5.78	5.48	
Oat plants	Decrease	5.67	5.65	Slight increase
Serradella tops	Increase	5.74	5.94	Decrease then increase
Timothy tops	Decrease	6.17	6.14	
		6.19	6.12	
Winter wheat tops	Decrease	6.33	5.95	
Winter wheat, leaves and stems, no heads	Decrease	6.12	5.77	

Table 4 gives the results of liming the soil upon the reaction of plant juices. Apparently most of the evidence at hand confirms Truog's suggestion that lime may function to decrease or regulate the actual acidity of the plant protoplasm.

The experiments of Kappen (13) emphasize the fact that as the actual acidity increases in the root juice, there is also a very appreciable increase in the total acidity of the juice. In the titration of the total acidities for limed and unlimed conditions, the writer found that when liming caused a decrease in the actual acidity, frequently there was a decrease in the total acidity; also that when liming produced an increased actual acidity, there was usually an increased total acidity. In this paper, the data for the effect of liming on the total acidities of plant juices are too meager to permit making a conclusive statement regarding it. That the total acidities of plant juices do sometimes fluctuate quite regularly in the same direction as the actual acidities, will be shown in a subsequent paper by Bauer and myself. Miss Irwin (16) has made the interesting observation that under certain conditions the carbon-dioxide production by petals may increase and still the actual reaction of the cell contents may decrease.

AGRICULTURAL PLANTS WITH HIGH ACTUAL AND TOTAL ACIDITIES

As stated the actual reaction of the juice of yellow lupines was found to be pH = 5.31 and pH = 5.63 for unlimed and limed conditions, respectively. Hempel (11) working with 20-day-old white lupine seedlings, found their actual reaction to be pH = 5.93. Of the plants with quite acid juices, buckwheat is of interest. It was mentioned before that the juices of buckwheat (entire seedlings 5 to 6 inches high with but few leaves on each seedling) had actual reactions of pH = 5.48 and pH = 5.97 when the plants were grown on unlimed and limed soil, respectively. Buckwheat tops, partially flowering and in seed, gave a juice with an actual acidity of pH = 4.82. An aqueous extract of the soil on which this buckwheat was grown had an actual reaction of pH = 7.68. The actual acidity of the juice of nearly mature buckwheat plants is markedly greater than during the early stages of growth. The titration values for the actual and the total acidities of the juice of buckwheat plants in their advanced stage are plotted in figure 5.

The curve for buckwheat indicates a marked buffer action of its juice. The 15-cc. sample of the centrifuged juice required the addition of about 4.2 cc. of the alkali to bring the juice to pH = 6.81, which is the color change for litmus, and about 8 cc. of the alkali to bring the juice to pH = 8.31, the color change for phenolphthalein.

VARIATIONS OF REACTION IN DIFFERENT PARTS OF PLANTS

The titration curves, in figure 4, for medium red clover, from limed and unlimed soils, show that the actual acidities and the acid reserves of the juices of the leaves, stems, and roots may differ quite considerably. By acid reserve we mean the amount of dissolved acid or acid salts in the undissociated form that dissociates as we neutralize some of the acid (hydrogen ions). The curves in figure 4 show that for both soils the leaf juice had the greatest acid reserve. The acid reserve of the root juice, however, was closer to that of the stems and petioles than to that of the leaves, and was somewhat intermediate in position. The results of Kappen (13) indicate that the juice of the above-ground parts of a plant has a greater total acidity than that of the roots.

The importance of determining the actual and total acidities of comparable tissues or organs, or parts of the plants, rather than of the entire tops, is further emphasized in the experiments with sweet clover. It is evident that if plant juice varies in its actual or total reaction according to the part of the plant from which it is expressed, then the number of leaves, stems, roots, etc., on the two lots of plants that are being compared may become a factor of considerable importance.

In order to study the variations of actual reaction in different parts of plants, the results obtained for blue-grass may be examined. The plants were about 20 inches high and in the pollinating stage. The soil reaction was neutral to slightly alkaline. The actual acidities of the uncentrifuged juices of the upper five inches of the tops which included all of the inflorescences, and of the lower 15 inches of the tops, were determined and found to be as follows: upper 5 inches of tops including inflorescences, pH = 6.11; lower 15 inches of tops, pH = 5.92. The results for blue-grass show that plant juice may vary in its actual reaction in different parts of the plant.

The results with orchard grass also show a change of actual reaction of the juice in different parts of the plant. The orchard grass was cut June 9 from a neutral to slightly alkaline soil. Determinations were made of the actual reaction of the different parts of the plants, using 10-cc samples of juice centrifuged for 10 minutes. The curves in figure 6 plotted from the results obtained, show that the initial actual acidity of the juice of the stems is greater than that of the leaves. Between pH = 6 and 9 the total acidity of the leaf juice is greater than that of the stem juice. Until approximately the point pH = 6 is reached, the conditions of total acidities are reversed from what they are beyond pH = 6.

IS THERE A GRADIENT OF REACTION IN PLANTS?

An extreme case, that of sweet clover, may be taken to emphasize the differences that exist in the reaction of the juice from different parts of the same plant. At 3:45 p.m., May 29, an entire sweet clover plant was dug up. The actual reaction of the soil extract was pH = 7.68. The tops were 24 inches high and the length of thickened root obtained was about 8 to 10 inches. The tops represented the second year's growth. The sunlight was intensely bright and the leaves had lost some of their turgidity. The actual acidity determinations of the juice taken from different portions of the same plant are given in table 5. These results indicate that the juice in this particular plant was acid, neutral, and alkaline, depending upon the portion of the plant from which the juice was taken. Among the numerous plants examined, the writer has not found another species exhibiting this condition. It is evident from table 5 that in this case a steep gradient existed in the actual reaction from the one end to the other, the upper end being the least acid. The studies of Child (2) on the metabolic and susceptibility gradients in plants and animals are of great interest, and possibly the use of sweet clover in these studies would give further valuable information. At present our results upon acidity gradients are too meager to permit of conclusions regarding their significance.

In connection with acidity gradients in plants it is well also to consider their relation to enzyme activity (3, 17), since the reaction within a plant may range from marked acidity at one end to a considerable alkalinity at the other end. Bunzell (1) has reported the results of a thorough study of the degree of oxidase activity of different plant juices, but has found it necessary to carry on all of the experiments in a solution, the actual acidity of which was greater than that of the neutral point for distilled water. In alkaline solutions, pyrocatechol, as well as most other good oxidase reagents, are oxidized by atmospheric oxygen, so that it is quite impossible to distinguish between the oxidation due to the alkalinity of the solution and that due to the oxidase activity of the plant juice.

In order to demonstrate conclusively that a large plant like this can possess an alkaline reaction of the degree found at one end of the acidity gradient, some second year's growth of sweet clover was cut from various locations about the Soils Building at the Wisconsin station. The entire tops of the various lots gave the following pH values: 7.50, 7.51, 7.54, 7.55, 7.59, 7.80, and 7.90. It is therefore evident that for plants like sweet clover, it is important to compare results from corresponding parts of plants. The different actual acidities of the different parts of sweet clover, together with the different proportions of leaves, stems and petioles in the various lots, undoubtedly have been largely responsible for the above wide range of 7.50–7.90 in the pH value.

THE ACTUAL REACTION OF THE JUICE FROM THE SUCCEEDING YEAR'S GROWTH OF THE SAME CROP

When the pH values, in table 5 are compared for the first and second year's growth of sweet clover, it is found that the different parts of the first crop of the first year's growth were somewhat more acid than the second year's growth. Further experiments are necessary before a conclusion can be reached in this regard.

Since the second year's growth was much more vigorous than that of the first year, the question arises: do the more vigorously growing plants of a species show a decrease in the actual reaction of their juice compared with that of less actively growing plants of the same species?

TABLE 5

Actual reaction of the juice from different portions of sweet clover plants

ACTUAL ACIDITY	DESCRIPTION OF MATERIAL USED
First crop; First year's growth	
pH	
6.53, 6.65, 6.68, 7.38	Each lot composed of leaves, petioles and buds
6.38, 6.46, 6.48, 6.48	Each lot composed of stems only
First crop; Second year's growth	
8.00	Upper 3 inches of the tops, stem, leaves and buds
7.04	Leaves and petioles of remainder of tops (no stems)
6.68	Stems to about 2 inches above the soil (no leaves)
6.46	2 inches of lower part of stem 2 inches of upper part of root
5.82	Root· 6-inch portion below the upper 2 inches of root

ACID RESERVE OF THE JUICE OF PLANTS GROWING IN SAME ENVIRONMENT

Since it has been observed that the juice of the second year's growth of sweet clover may be markedly alkaline in reaction, it may be of interest to compare the acid reserve of the juice from the tops of the second year's growth of sweet clover with that of the second year's growth of medium red clover. Both species were growing together in a plot, an aqueous extract of which gave a reaction of pH = 7.51. The plants were cut May 23.

Determinations were also made of the acid reserve of the root juice of the two species of plants which were obtained from the same part of the plot 3 days later. Ten-cubic-centimeter samples of juice centrifuged for 15 minutes were used in every case. The titrations were repeated on a second series of aliquots of the same juice and the results checked very closely. The results are shown in figure 7.

The curves indicate that the juice of the roots in the two cases has a greater acid reserve than the juice of the tops of similar plants cut 3 days previously. They indicate also that the juice of the medium red clover tops and of the roots has a greater acid reserve than that of the sweet clover tops and roots, respectively. Furthermore, the actual acidity of the juice of medium red clover tops is greater than that of sweet clover, tops, whereas the actual acidity of the juice of the roots of both species was practically the same.

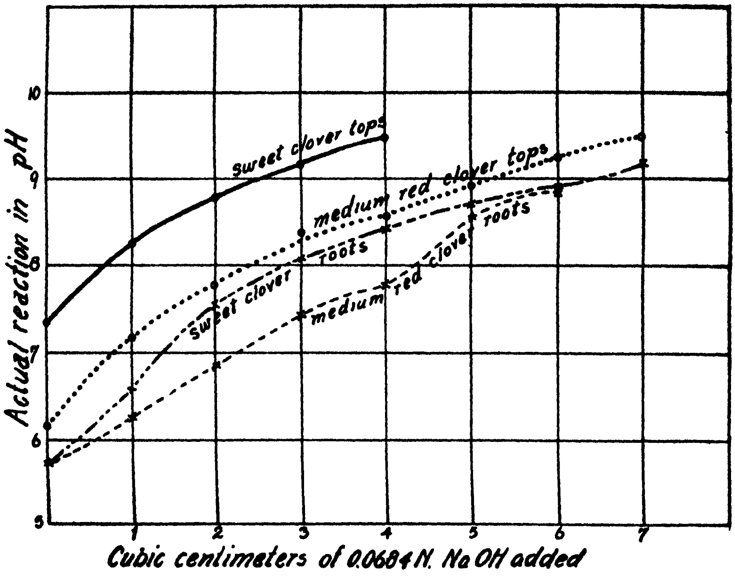

FIG. 7. CURVES SHOWING THE ACID RESERVE OF TOPS OF THE SECOND YEAR'S GROWTH OF SWEET CLOVER AND OF MEDIUM RED CLOVER CUT ON THE SAME DAY, AND THAT OF THE ROOTS OF BOTH SPECIES OBTAINED 3 DAYS LATER

THE ALKALI RESERVE OF PLANT JUICE

It was considered worth while to determine the degree to which the juice of the second year's growth of sweet clover could become alkaline. A group of such plants 24 to 34 inches high was growing luxuriantly near the edge of a burned refuse pile. The reaction of the soil extract was pH = 7.68. The uppermost 2 inches of a large number of the plants were plucked and only the very tip of the axis, together with its unfolding leaves, was retained for the determination of the actual reaction of the juice.

When the tips were cut the sun had been shining several hours, although the previous two days it had been raining. The juice was centrifuged 5 minutes and the actual reaction was found to be pH = 8.47. Since phenolphthalein turns pink at pH = 8.31, the juice of the tips of sweet clover had an actual reaction apparently alkaline to phenolphthalein. The alkali reserve of a 10-cc. portion of centrifuged juice of the tips of sweet clover was then determined by adding different amounts of standard acid to the juice and observing the new pH after each addition of the acid.[5] The values obtained in this way are given in the form of a curve in figure 8.

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[5] By alkali reserve we mean the change in the actual reaction of the juice upon the addition of acid.

Since sweet clover juice was decidedly alkaline and had a high alkaline reserve, it appeared probable that it might contain more carbonates than the more acid juice of medium red clover tops. The apparatus of Van Slyke (26) was used for the estimation of carbon dioxide in the juices.

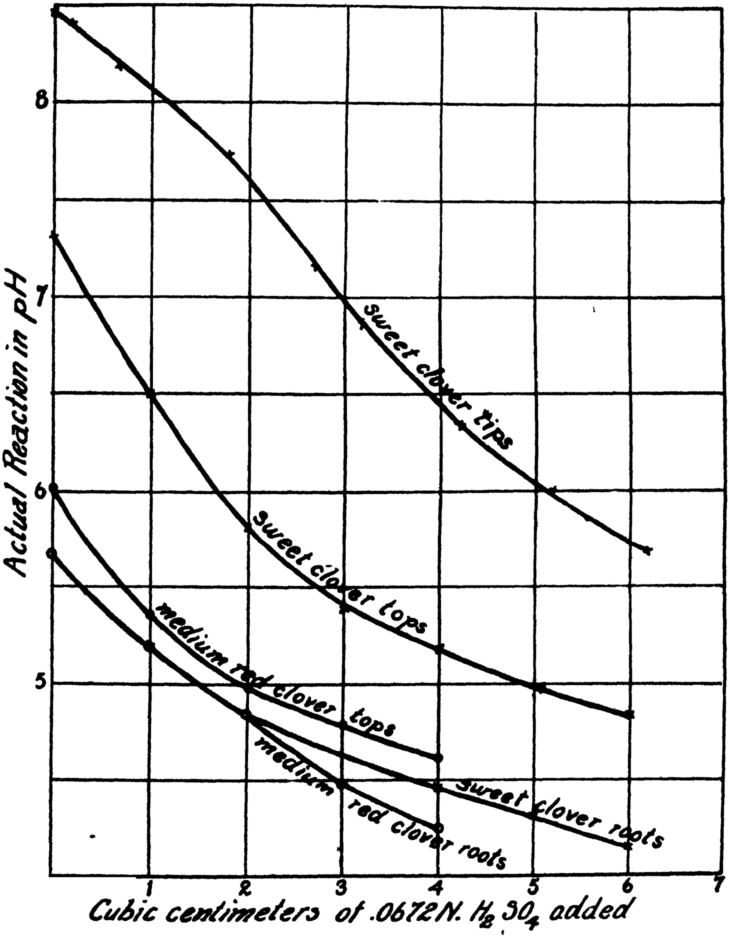

FIG. 8. CURVES SHOWING THE ALKALI RESERVE OF DIFFERENT PARTS OF SWEET CLOVER AND MEDIUM RED CLOVER

The readings of table 6 are not intended for absolute values, and hence corrections have not been made. The juice of sweet clover tops contained approximately 50 per cent more carbon dioxide than did that of medium red clover. Undoubtedly some of the carbon-dioxide gas existed as dissolved gas in the juice, and hence without further experiments it is impossible to say how much was in the carbonate or bicarbonate form. It is for this reason that the results for medium red clover are given as a control. The results for the two species may therefore serve as a rough comparison of the relative amounts of carbonates and bicarbonates in the juice of the two species.

The juice of the roots of the second year's growth of sweet clover was then examined for its alkali reserve. The juice from the tops of the plants, from which the roots were taken, had an actual acidity of pH = 7.90. Likewise the actual reaction and alkali reserve of the juice of medium red clover roots from the same plot as that from which the sweet clover roots were taken were then determined. The alkali reserve of the juice of the tops of both species from the same plot as before also was then determined. Ten-cubic-centimeter samples of juice were used in the several titrations. The actual acidity and alkali-reserve determinations were carried on at about the same time, since it has been shown especially for some of the succulents that a considerable diurnal variation may take place in the reaction of the juice (14, 15, 18, 19 and 22). From the curves (fig. 8) plotted from the results obtained, it is seen that the juice of the unfolding tips of sweet clover has a much greater alkali reserve than that of the tops, and that of the tops greater than that of the roots. The alkali reserves of the root juices of sweet and of medium red clover are practically identical until pH = 4.85 is reached, at which point the curves begin to diverge. The alkali reserve of the juice of medium red clover tops is greater than that of the roots, though the difference is not as marked as with sweet clover. Much more data are necessary before any far-reaching conclusions can be drawn.

TABLE 6

Total amount of carbon dioxide (uncorrected) in 3-cc. aliquots of the uncentrifuged juice of tops

SWEET CLOVER	MEDIUM RED CLOVER
cc.	*cc.*
0 142	
0 140	0 090
0 170	0 090
0 140	0 100
0 180	

EFFECT OF THE ABSENCE OF CHLOROPHYLL (INHERITABLE) UPON THE REACTION OF PLANT JUICE

In studies upon normal and etiolated lupine seedlings Hempel (11) found that light decreases the total acidity to litmus, but increases the total acidity to phenolphthalein when compared with the total acidity of plants kept in the dark, although the actual acidity remains nearly constant. The writer was able to secure corn seed that produced seedlings, about 25 per cent of which were pure white.[6] The juice of both the green and the colorless plants was tested for its actual and total acidity.

A preliminary experiment was carried on, using other corn seedlings. The results of the preliminary experiment are not to be emphasized on account of the fact that it is uncertain whether or not all of the seedlings received similar conditions of illumination.

The seedlings, of which only the tops were used, were 18 days old when the preliminary experiment was carried on. The actual and total acidities of 6-cc. samples of juice that had been centrifuged 15 minutes were determined. If the curves in figure 9 are examined it is found that the juice of the chlorophylless tops did not behave in a manner similar to that of the chlorophyllous tops as regards total acidity. The total acidity of the juice of the green tops is much greater than that of the white tops until about the point pH = 8.7 is reached, when the curves cross each other. Moreover, the initial actual acidity of the juice of the green tops was greater than that of the white tops. If the chlorophylless tops behaved like etiolated plants, then, according to the interesting observations of Hempel (11) for etiolated lupine seedlings, it should be expected that the chlorophylless tops would have shown a greater total acidity than the chlorophyllous tops to litmus—pH = 6.81, but a smaller total acidity than the chlorophyllous tops to phenolphthalein—pH = 8.31. Neither of these two conditions hold in the curves

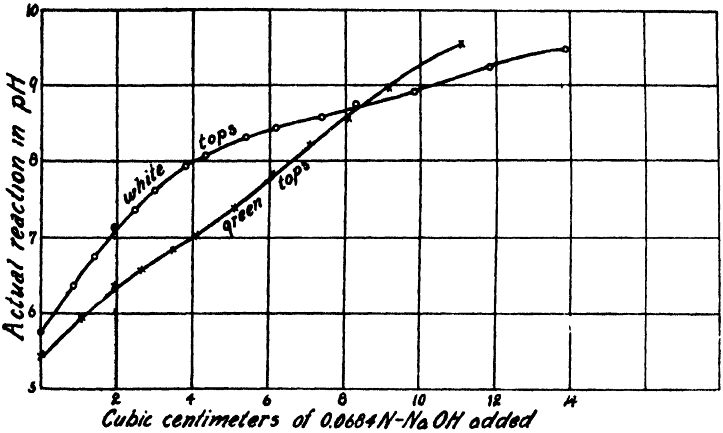

FIG. 9. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF GREEN AND OF WHITE TOPS OF CORN SEEDLINGS

given; hence, it appears from this preliminary experiment, that as regards total acidity the chlorophylless plants do not behave as though they were etiolated.

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[6] These were furnished through the kindness of E. W. Lindstrom, of the University of Wisconsin.

Another similar experiment was carried out in which the different seedlings were grown among one another for 10 days and were given similar environmental conditions. The titrations were made on 10-cc. samples of juice that had been centrifuged 10 minutes.

The results of the determinations are given as curves in figure 10. The curves show much the same results as were observed in a preliminary experiment, and given in figure 9.

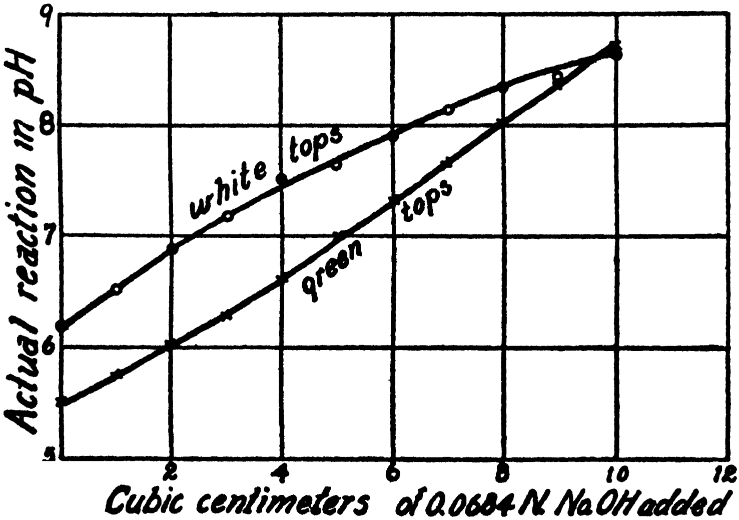

FIG. 10. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF GREEN AND OF WHITE TOPS OF CORN SEEDLINGS

Among the corn seedlings a number of the white tops were tinted somewhat green. As expected, their pH value was intermediate between that obtained for pure white and for green tops. The actual values found were as follows: green tops pH = 5.52, green-tinted white tops pH = 5.85, and white tops pH = 6.16.

EFFECT OF LIGHT AND AGE ON THE REACTION OF THE JUICE OBTAINED FROM PLANTS

Corn seed from a single ear that produced all green seedlings was planted June 3. Half of the seeded area was covered with a large box that was covered with black paper. At the end of 10 days the seedlings with green tops were 5 inches high, whereas the greenish-yellow tops of the darkened seedlings were 9 inches high. Some of the corn seedlings with green tops were placed in a subcellar in total darkness from June 13 until June 16. Determinations were made of the actual and total acidities of the tops of both the 10- and 13-day-old plants, respectively, using in every case 10-cc. samples of juice that had been centrifuged 10 minutes.

On plotting the data obtained for the 10- and 13-day corn seedling tops (fig. 11), it is found that the total acidity of the juice of the 13-day green tops was greater than that of the 10-day green tops for any pH taken as the end-point. This is in accord with the results of Hempel (11) on white lupine seedlings in which she found that for normal seedlings the total acidity of the juice (taking litmus pH = 6.81 and phenolphthalein pH = 8.31 as end-points) increased with the increasing age of the seedlings. Undoubtedly the 10-day darkened tops must have received very faint illumination, for otherwise the tops would have been more blanched.

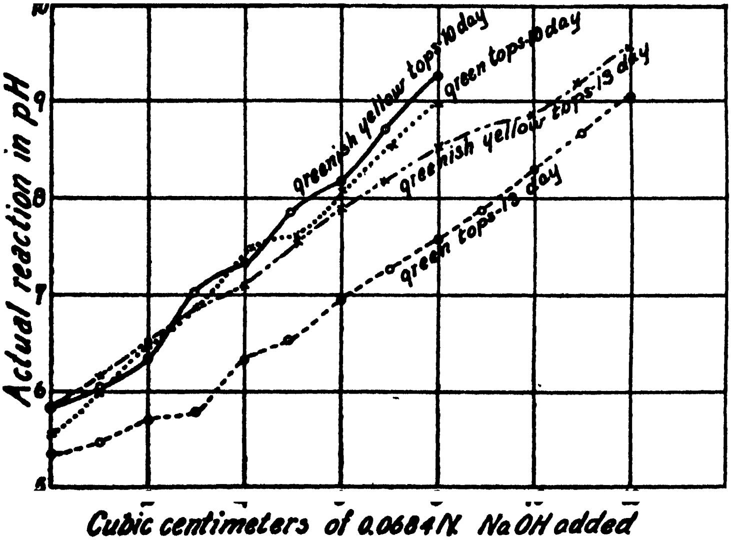

FIG. 11. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF GREEN AND OF GREENISH-YELLOW TOPS OF CORN SEEDLINGS

For the 10-day seedlings, the initial actual acidity of the darkened tops is less than that of the undarkened tops. The curves for the total acidity of the juices of the two kinds of 10-day-old tops lie very close to one another, but show to a less marked degree the same results as the 13-day-old tops. The close approach to each other of the curves for the 10-day tops may be the result of an insufficient degree of etiolation or may be due to the young age of the seedlings.

When the green seedlings were permitted to remain 10 days in the light and part were then placed in total darkness for 3 days, it is found as before that the initial actual reaction of the juice of the tops exposed 13 days to the light was greater than that of the tops exposed 10 days to the light and 3 days to total darkness. The total acidity of the juice of the tops darkened for 3 days is much less than that of the undarkened tops.

SUMMARY

The actual and total acidities and alkali reserve of a number of agricultural plants have been determined for certain conditions of growth. In order to ascertain the actual reaction of minute quantities of plant juice, a hydrogen-electrode vessel has been used that gave excellent results with only 3 to 4 drops of juice.

The reaction of the juice of a plant appears to be affected by changes in illumination, soil solution, age, and other conditions, indicating that the reaction of plant juice is the resultant of many processes. The results of preliminary experiments bearing upon some of these factors are herein reported.

The juice of buckwheat seedlings was found to possess an actual acidity of pH = 5.48 to 5.97, whereas in the mature condition a reaction of pH = 4.82 was reached. It appears that during the life cycle of buckwheat plants the juice may undergo a marked change in its actual reaction. The degree of maturity of a plant seems to be an important factor in the degree of actual acidity attained. Table 4 shows that the actual reaction of the juice of many agricultural plants is distinctly acid.

In order to obtain a measure of the buffer action of plant juice, determinations have been made of the acid and alkali reserve of the juice of certain plants. The alkali reserve of the roots of medium red clover and of sweet clover (fig. 8) is less than that of the corresponding tops. The acid reserve of the roots of medium red clover (fig. 4) is less than that of the leaves, even though the actual acidity of the roots is greater than that of the leaves. The unique case of a single plant having juice with a markedly alkaline actual reaction and a steep gradient in actual reaction is reported. The variations in the actual reaction of plant juice in the various portions of the plant have been investigated.

Although the determinations of the total acidity (table 4) have been too few in number, those reported, together with further observations to be presented later, indicate the existence of a close relation between the actual and the total acidity, and appear to show that the total acidity tends to fluctuate in the same direction as the actual acidity, although exceptions may frequently occur. It has been pointed out that the proportion of stems, leaves, petioles, etc., on plants, may influence the values obtained for the actual and the total acidities of the juice. The juice of the younger portions of a plant usually has a lower actual acidity than the older portions.

The total acidity of the juice of chlorophylless (inheritable) corn seedling tops is less than that of chlorophyllous tops until about pH = 8.6 is passed, after which the reverse is true. The actual and the total acidity of the juice of corn seedling tops increases with the increasing age of the seedlings and decreases with a reduction of the normal illumination.

There is considerable experimental data in favor of the suggestion of Truog that the main specific harmful influence of soil acidity on certain plants is due to its influence in preventing the plants from securing rapidly enough the bases that are needed to neutralize and precipitate acids within the plant. Further experiments under controlled conditions will be necessary before all the factors can be fully understood.

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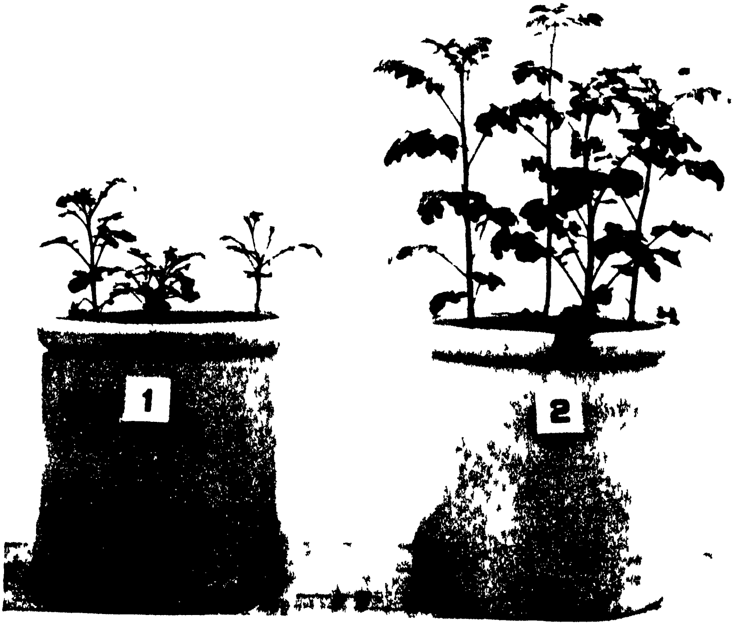
PLATE 1

EFFECT ON THE GROWTH OF WHITE MUSTARD PLANTS OF THE ADDITION OF LIME TO PLAINFIELD SAND: 1, LIMED; 2, NOT LIMED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FIELD EXPERIMENTS ON THE AVAILABILITY OF NITROGENOUS FERTILIZERS, 1908–1917[1]

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In 1908 field experiments were started for the purpose of studying the relative availability of different nitrogenous fertilizer materials. The plan was developed somewhat along the lines followed for the cylinder experiments which had been started ten years before.

In the field experiments a larger number of nitrogenous materials was included and certain other variations were introduced. Lime was used on one section of the nitrogen-treated plots; another section of the plots with parallel nitrogen treatment was left without lime. The plan also provided for the study of denitrification problems.

The results of this work for the first 5 years have been published (2) and a brief summary of the second 5 years' work, so far as it relates to the lime treatment, also has appeared (1).

PLAN OF THE EXPERIMENT

For convenience the plan of the experiment may be briefly restated.

The plots are 1/20 acre in size, being separated by a 4-foot space, with a 10-foot roadway separating the two sections. The soil is a loam or gravelly loam and had not been under cultivation for some years previous to 1908.

Twenty of the plots designated as 1A to 20A have received no lime treatment during the ten years and 20 others designated as 1B to 20B received one ton of ground limestone per acre just before planting the corn in 1908, and a second application of 2 tons per acre preceding the corn crop of 1913.

Certain plots in each section receive no fertilizer, others receive one element only, others a combination of the two elements—phosphorus and potassium—and still others the three elements—nitrogen, phosphorus and potassium.

All nitrogen-treated plots receive annually 640 pounds of acid phosphate and 320 pounds of muriate of potash per acre. These are rather heavy applications but they are made in this way so that a deficiency of these materials may not become a limiting factor.

Stable manure has been applied to certain plots at the rate of 16 tons per acre annually and one plot in each section has received this amount of manure in addition to 320 pounds of nitrate of soda. One plot receives alfalfa hay, cut fine, at the rate of 2 tons per acre and another rye straw prepared in the same way and at the same rate per acre. These are used to furnish nitrogen in the form which would be furnished by crop residues or green manures. The latter however, cannot be grown on the plots where they are used, since the growing of alfalfa would complicate the nitrogen-availability studies.

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[1] Technical Paper No. 1 of the New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

The following plan gives the fertilizer treatment for each 1/20-acre plot in the two sections:

PLOT NUMBER		FERTILIZER TREATMENT
1A,	1B	Nothing
2A,	2B	16 pounds muriate of potash
3A,	3B	32 pounds acid phosphate
4A,	4B	*Minerals only
5A,	5B	Minerals, 1600 pounds cow manure
6A,	6B	Minerals, 1600 pounds horse manure
7A	7B	Nothing
8A,	8B	Minerals, 8 pounds $NaNO_3$
9A,	9B	Minerals, 16 pounds $NaNO_3$
10A,	10B	Minerals, $Ca(NO_3)_2$ equivalent to 16 pounds $NaNO_3$
11A,	11B	Minerals, $(NH_4)_2SO_4$ equivalent to 16 pounds $NaNO_3$
12A,	12B	Minerals, $CaCN_2$ equivalent to 16 pounds $NaNO_3$
13A,	13B	Minerals, dried blood equivalent to 16 pounds $NaNO_3$
14A,	14B	Minerals, fish equivalent to 16 pounds $NaNO_3$
15A,	15B	Minerals, concentrated tankage equivalent to 16 pounds $NaNO_3$
16A,	16B	Minerals, 200 pounds alfalfa hay
17A,	17B	Minerals, 200 pounds wheat or rye straw
18A,	18B	Minerals, 1600 pounds cow manure and 16 pounds $NaNO_3$
19A,	19B	Minerals only
20A,	20B	Minerals, 200 pounds wheat or rye straw and 16 pounds $NaNO_3$

* Minerals—32 pounds acid phosphate and 16 pounds muriate of potash

Fertilizers and manures are applied annually about the time of seeding the crops, with the exception that in the case of fall-sown crops, one-fourth of the mineral nitrogenous fertilizer is applied at the time of seeding and three-fourths as a top-dressing the following spring.

The results for the second 5-year period may be discussed in connection with the results for the first 5 years under the following headings:

(a) Yields of dry matter.
(b) Total yield of nitrogen.
(c) Percentage of nitrogen in the dry matter.
(d) Percentage of nitrogen recovered.
(e) Income and outgo of nitrogen.

YIELD OF DRY MATTER

The yields of dry matter—grain and straw or other roughage—for the 5 years, are shown in table 1.

It will be noted that this table is divided into two sections; the first constituting plots 1A to 20A (the unlimed section) and the second constituting plots 1B to 20B (the limed section). The results are reported in pounds per acre.

Plots 1 and 7 in each section have received no fertilizers. Plots 2 and 3 have received muriate of potash and acid phosphate, respectively, and plots 4 and 19 have received a combination of the two. These plots (4 and 19), therefore, constitute the checks, having received the minerals the same as the other plots, but no nitrogen.

An examination of the table shows that plot 1A has produced much better crops than plot 7A although neither receives any fertilizer. It may also be pointed out in this connection, that plots 2A and 3A have yielded more than the average shown for the check plots. This, taken in connection with the data for plot 7A, seems to indicate that the part of the field where these plots lie is better than the remainder of the section. There is at this point a slight depression and no doubt these plots have received some washings from the slightly higher points. However, so far as the nitrogenous fertilizer treatments are concerned, these plots may be eliminated from the discussion, as may also plot 7.

The influence of the nitrogenous fertilizers

The maximum yields in each section were secured on plot 18 which receives the 16 tons of manure and 320 pounds of nitrate of soda per acre. The yields from this plot are followed closely by plots 5, 6 and 20 in the two sections. It must be remembered, however, that on all of these plots excessive amounts of nitrogen have been used and it does not necessarily follow that the crops which they have yielded have been the most profitable. Indeed, in a number of cases, the excessive amount of nitrogen has caused lodging of the grain so that there was much loss at the time of harvesting. It is evident, also, that there has been a large loss of nitrogen from the soil on these plots. These points will be further discussed under the subject of "Income and Outgo of Nitrogen."

Plot 8 in each section receives one-half the standard nitrogen application, and it will be noted that the total yield on 8A is distinctly below the yields from plots that receive the full nitrogen application. The total yield on 8B, however, does not fall very much behind the total for those plots that receive the full nitrogen application. It would appear that for the crops grown the 160-pound application is not quite sufficient for a maximum yield.

The full application of nitrate of soda on plot 9A gave a total grain yield of 4065 pounds as against 3410 pounds for nitrate of lime, 3380 pounds for

TABLE 1

Yield of dry matter for the 5 years 1913–1917

PLOT NUMBER	CORN, 1913			OATS, 1914*		WHEAT, 1915		TIMOTHY		TOTAL GRAIN	TOTAL HAY, STRAW, ETC.	INCREASE OVER CHECK	
	Grain	Stalks	Cobs	Grain	Straw	Grain	Straw	1916	1917			Grain	Hay, straw, stalks, etc.
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1A	850	1,500	175	540	760	940	1,160	1,920	1,380	2,330	6,895		
2A	800	1,550	150	660	780	760	1,080	2,080	1,120	2,220	6,760		
3A	700	1,600	100	800	880	980	1,220	1,800	1,080	2,480	6,680		
4A	650	1,775	75	740	940	940	1,200	1,580	900	2,330†	6,470†		
5A	2,200	2,525	325	720	1,660	1,820	3,160	4,160	4,560	4,740	16,390	2,592	10,332
6A	2,250	2,950	325	680	1,740	1,880	3,760	4,620	4,500	4,810	17,895	2,662	11,837
7A	400	875	75	480	400	280	400	640	400	1,160	2,790		
8A	875	1,525	100	700	980	1,120	1,500	2,560	2,060	2,695	8,725	547	2,667
9A	1,625	1,975	225	700	1,200	1,740	2,520	3,880	3,880	4,065	13,680	1,917	7,622
10A	1,250	1,625	200	620	1,200	1,540	2,400	3,700	4,040	3,410	13,165	1,262	7,107
11A	1,300	1,650	175	800	1,400	1,280	1,800	1,600 880‡	1,920	3,380	9,425	1,232	3,367
12A	1,450	2,050	200	760	1,080	1,460	2,120	2,640	2,500	3,670	10,590	1,522	4,532
13A	1,325	1,950	225	560	1,160	1,420	2,160	2,800	2,400	3,305	10,695	1,157	4,637
14A	1,625	2,175	250	560	720	1,520	2,280	2,840	3,640	3,705	11,905	1,557	5,847
15A	1,750	2,250	225	340	880	1,300	1,520	2,440	2,520	3,390	9,835	1,242	3,777
16A	2,000	2,400	275	500	1,120	1,540	2,320	3,300	3,440	4,040	12,855	1,892	6,797
17A	1,075	2,000	125	460	640	800	1,080	2,360	1,480	2,335	7,685	187	1,627
18A	2,600	2,975	425	600	1,520	2,360	3,960	4,480	4,220	5,560	17,580	3,412	11,522
19A	625	1,800	125	600	740	740	800	1,420	760	1,965†	5,645†		
20A	1,950	2,400	250	660	1,200	1,740	2,680	4,240	4,440	4,350	15,210	2,202	9,152
Average	1,365	1,978	201	624	1,050	1,308	1,956	2,797	2,562	3,297	10,544		

1B..........	725	1,375	150	580	620	680	840	1,400	700	1,985	5,085		
2B..........	1,250	1,425	175	560	720	720	940	1,680	800	2,530	5,740		
3B..........	925	1,550	225	540	680	680	760	1,880	920	2,145	6,015		
4B..........	1,550	2,350	175	340	640	620	800	1,780	1,100	2,510†	6,845†		
5B..........	2,175	2,800	325	Sample lost		1,520	2,640	3,300	3,880	3,695	12,945	1,237	5,505
6B..........	1,650	1,725	250	460	1,400	1,680	3,160	3,280	3,500	3,790	13,315	1,332	5,875
7B..........	1,575	1,875	275	220	720	680	960	2,000	840	2,475	6,670	17	
8B..........	2,025	2,100	300	660	1,080	1,100	1,560	2,840	2,000	3,785	9,880	1,327	2,440
9B..........	1,975	2,300	350	300	1,080	1,280	1,880	3,480	3,060	3,555	12,150	1,097	4,710
10B..........	2,375	2,650	325	480	1,340	1,660	2,400	4,200	3,480	4,515	14,395	2,057	6,955
11B..........	2,550	2,725	375	500	1,260	1,600	2,480	4,080	3,540	4,650	14,460	2,192	7,020
12B..........	2,225	2,850	325	400	1,280	1,500	1,960	3,500	2,200	4,125	12,115	1,667	4,675
13B..........	2,225	2,700	325	580	1,120	1,400	2,140	3,420	2,240	4,205	11,945	1,747	4,505
14B..........	2,250	2,675	325	520	1,080	1,260	1,880	3,000	2,140	4,030	11,100	1,572	3,660
15B..........	2,350	2,425	350	520	980	1,080	1,720	3,040	2,200	3,950	10,715	1,492	3,275
16B..........	2,200	2,575	350	380	1,180	1,340	2,420	3,240	3,120	3,920	12,885	1,462	5,445
17B..........	2,600	2,525	375	520	660	1,140	1,400	2,900	3,220	4,260	11,080	1,802	3,640
18B..........	2,950	2,625	425	600	2,060	2,320	4,120	4,160	4,560	5,870	17,950	3,412	10,510
19B..........	1,025	2,300	175	480	680	900	1,160	2,000	1,720	2,405†	8,035†		
20B..........	2,425	2,625	400	820	1,320	1,940	2,880	4,280	4,220	5,185	15,725	2,727	8,285
Average.....	1,951	2,309	299	498	1,047	1,255	1,905	2,973	2,472	3,679	10,953		

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* The oats crop was damaged by heavy rains at harvest time.

† Check.

‡ Second growth—largely crab grass.

sulfate of ammonia and 3670 pounds for calcium cyanamid. The full application of nitrate on 9B, on the other hand, gave a total yield of 3555 pounds of grain as against 4515 pounds for calcium nitrate, 4650 pounds for ammonium sulfate and 4125 pounds for calcium cyanamid.

Seven of the plots 9 to 15, inclusive, in each section receive equivalent amounts of nitrogen and it is of interest to compare the average yields from these plots with the average from the two check plots. These figures are shown in table 2.

In all cases the nitrogen-treated plots show a substantial increase over the check plots. In most cases this increase amounts to more than 50 per cent, which means that the outlay for nitrogen has proven a good investment. It is quite possible, however, that had the application of nitrate been less than 320 pounds—200 or 250 pounds per acre—the increase in yield might have been as great, which would have meant a larger profit than was obtained with the 320 pounds.

TABLE 2

Average yield of dry matter—check plots compared with nitrogen-treated plots

		UNLIMED SECTION		LIMED SECTION	
		1908–1912	1913–1917	1908–1912	1913–1917
		lbs.	*lbs*	*lbs*	*lbs*
Check	Grain	4,544 4	2,147 5	3.907 5	2,457 5
	Straw, etc .	9,044 8	6,057 5	8,818 7	7 440 0
Seven nitrogen treated plots	Grain	5,831 2	3,560 7	6,248 9	4,147 1
	Straw, etc .	14,179 4	11,328 0	14,275 6	12,411 4

It will be noted that the average yields for the second 5-year period are less in all cases than those for the first 5-year period. This is partly due to the fact that a residual crop of corn was grown after the oat crop of 1909. Such residual crop was not grown in the second 5-year period.

Four of the seven plots referred to above receive mineral nitrogenous materials and three receive organic materials in equivalent amounts. We may, therefore, compare the yields obtained from plots receiving the organic materials with those from plots receiving the mineral materials. This comparison is made for both sections and for the two 5-year periods in table 3, the figures being the average for three plots in one case and for four in the other.

An examination of this table shows that in all cases, the yields of both grain and straw, stover, etc., are larger where the mineral nitrogenous materials were used than with the organic materials. This is in agreement with results obtained in cylinder experiments, reference to which has already been made.

It has been thought by some, that the residual effect of organic materials would outweigh the quicker effect of readily soluble materials. It does not appear to be so. The readily soluble materials give the plant a good start at a time when the nitrification of organic matter is slow and this good start apparently keeps the plant in the lead to the end. With such a start, it is better able to use soil moisture and the natural plant-food than is the plant which gets a slower start. Furthermore, it is very probable that during the time required for the transformation of the organic nitrogen into available plant-food the loss from this is greater than the loss by leaching from the mineral compounds.

Whatever reason may be assigned for the difference, it is very evident that a pound of nitrogen from the mineral materials is more efficient than a pound from the organic materials. This statement is made on the basis of results secured during ten years of field experiments and twenty years of cylinder experiments.

TABLE 3

Average yield of dry matter—mineral and organic nitrogenous materials compared

		UNLIMED SECTION		LIMED SECTION	
		1908–1912	1913–1917	1908–1912	1913–1917
		lbs.	*lbs*	*lbs.*	*lbs*
Average four mineral materials	Grain	6,002.5	3,631.2	6,379.6	4,211.2
	Straw, etc ...	14,686.4	11,715.0	15,534.6	13,280.0
Average three organic materials	Grain	5,602.9	3,466.7	6,074.5	4,061.7
	Straw, etc .	13,503.4	10,812.0	12,596.8	11,253.3

The yields from plots 16 and 17 are of interest. It will be recalled that plot 16 received alfalfa hay (cut fine) at the rate of 2 tons per acre and plot 17 rye straw prepared in the same way and applied at the same rate per acre. Naturally, a given amount of alfalfa furnishes more nitrogen than an equal amount of the straw, and furthermore, it is in a more available form. It is not surprising therefore, to find the yields from 16A considerably larger than those from 17A. In an acid soil, the straw decomposes very slowly and therefore yields very little available nitrogen.

On the other hand, the 5-year yield from 17B is larger than the yield from 16B. This is probably due to the fact that for five or six years volunteer clover and vetch have been coming into 17B. The lime and minerals which are applied on this plot make the conditions favorable for the legumes and as a result, the latter flourish at the expense of grass and grain. On 16B the alfalfa hay on a well limed soil furnishes enough available nitrogen to encourage the growth of the grass and grain crops to the exclusion of volunteer legumes.

TABLE 4

Yield of nitrogen for the 5 years 1913–1917

PLOT NUMBER	CORN, 1913				OATS, 1914			WHEAT, 1915			TIMOTHY		NITROGEN IN		TOTAL NITROGEN
	Grain	Stalks	Cob	Total	Grain	Straw	Total	Grain	Straw	Total	1916	1917	Grain	Hay, Straw, Stalks, etc.	
	lbs	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*
1A	12 95	16 89	1 00	30 84	10 88	5 70	16 58	19 34	4 11	23 45	17 51	13 32	43 17	58 53	101 7
2A	11 30	11 16	0 83	23 29	12 78	5 70	18 48	15 78	4 46	20 24	21 44	11 47	39 86	55 06	94 9
3A	10 23	13 28	0 74	24 25	15 54	5 96	21 50	20 34	3 84	24 18	17 08	9 89	46 11	50 79	96 9
4A	8 87	13 86	0 30	23 03	14 92	5 62	20 54	18 96	4 61	23 57	14 84	8 51	42 75	47 74	90 5*
5A	34 98	20 91	1 35	57 24	17 20	15 08	32 28	35 27	13 68	48 95	31 49	35 48	87 45	117 99	205 4
6A	33 57	25 37	1 19	60 13	16 72	19 22	35 94	39 41	17 41	56 82	37 93	36 36	89 70	137 48	227 2
7A	6 08	7 08	0 53	13 69	8 82	3 06	11 88	5 84	1 50	7 34	6 53	3 74	20 74	22 44	43 2
8A	12 01	9 32	0 44	21 77	15 04	7 70	22 74	19 94	5 18	25 12	18 69	15 02	46 99	56 35	103 3
9A	25 04	12 46	1 22	38 72	16 98	11 66	28 64	34 22	11 16	45 38	26 19	28 29	76 24	90 98	167 2
10A	19 38	16 35	1 17	36 90	14 10	10 48	24 58	30 60	10 39	40 99	25 64	27 84	64 08	91 87	156 0
11A	19 77	13 51	0 85	34 13	18 68	13 60	32 28	29 98	12 22	42 20	25 77	19 85	68 43	85 80	154 1
12A	19 49	14 56	0 97	35 02	17 34	9 14	26 48	30 62	8 56	39 18	23 13	22 40	67 45	78 76	146 2
13A	19 64	14 04	1 20	34 88	11 48	8 90	20 38	28 64	10 20	38 84	26 29	20 57	59 76	81 20	141 0
14A	27 30	16 10	1 21	44 61	12 54	6 04	18 58	28 10	10 53	38 63	24 34	28 32	67 94	86 54	154 5
15A	26 11	18 63	0 96	45 70	7 72	8 32	16 04	25 83	6 28	32 11	22 91	21 85	59 66	78 95	138 6
16A	34 58	28 42	1 07	64 07	12 30	11 08	23 38	32 56	8 68	41 24	27 98	28 10	79 44	105 33	184 8
17A	17 63	14 80	0 91	33 34	9 60	4 86	14 46	17 39	4 68	22 07	20 67	13 41	44 62	59 33	104 0
18A	43 94	27 28	1 68	72 90	14 72	15 16	29 88	53 41	18 73	72 14	38 39	41 99	112 07	143 23	255 3
19A	8 83	11 02	0 88	20 73	12 04	5 02	17 06	15 64	3 07	18 71	12 30	7 41	36 51	39 70	76 2*
20A	32 76	21 77	1 43	55 96	15 00	11 02	26 02	36 96	14 50	51 46	34 43	32 37	84 72	115 52	200 2
Average	21 22	16 34	1 00	38 56	13 72	9 17	22 89	26 94	8 69	35 63	23 68	21 26	61 88	79 66	142 0

1B	11 60	12 35	0 70	24 65	12 30	5 02	17 32	13 45	3 23	16 68	14 56	6 62	37 35	42 48	79 8
2B	18 28	14 62	0 92	33 82	11 48	5 20	16 68	15 03	3 23	18 26	17 93	7 64	44 79	49 54	94 3
3B	14 71	14 83	1 22	30 76	11 42	5 82	17 24	14 47	2 69	17 16	19 72	8 06	40 60	52 34	92 9
4B	20 49	18 54	0 78	39 81	6 88	4 56	11 44	13 37	2 99	16 36	15 41	9 54	40 74	51 82	92 6*
5B	35 24	26 52	1 35	63 11	†	†	†	30 42	10 40	40 82	31 32	32 09	65 66	101 68	167 3
6B	26 90	16 68	1 26	44 84	10 54	13 60	24 14	39 65	14 60	54 25	34 41	31 86	77 09	111 91	189 0
7B	25 20	19 05	1 14	45 39	3 94	4 76	8 70	14 07	4 63	18 70	20 24	7 28	43 21	57 10	100 3
8B	31 21	20 92	1 48	53 61	14 94	8 66	23 60	20 94	6 15	27 09	22 81	15 76	67 09	75 78	142 9
9B	31 60	20 19	1 80	53 59	7 24	11 36	18 60	26 11	7 95	34 06	28 57	23 81	64 95	93 68	158 6
10B	36 34	18 82	1 32	56 50	11 56	13 38	24 94	33 70	8 74	42 44	32 93	26 03	81 62	101 22	182 8
11B	37 79	23 93	1 70	63 42	11 94	12 80	24 74	33 28	11 24	44 52	33 86	26 83	83 01	110 36	193 4
12B	35 18	20 26	1 38	56 82	9 16	12 32	21 48	31 05	6 94	37 99	34 16	21 89	75 39	96 95	172 3
13B	34 29	21 09	1 48	56 86	13 80	10 78	24 58	28 56	7 15	35 71	28 39	19 85	76 65	88 74	165 4
14B	34 67	18 75	1 35	54 77	12 14	9 04	21 18	26 45	7 03	33 48	28 17	20 03	73 26	84 37	157 6
15B	36 92	25 34	1 35	63 61	12 46	10 30	22 76	21 83	5 75	27 58	27 18	19 71	71 21	89 63	160 8
16B	35 42	29 97	1 49	66 88	9 38	12 30	21 68	29 29	8 32	37 61	27 48	28 27	74 09	107 83	181 9
17B	44 69	25 91	1 93	72 53	10 62	4 48	15 10	24 70	5 78	30 48	25 40	37 09	80 01	100 59	180 6
18B	46 91	25 38	1 81	74 10	14 98	20 92	35 90	54 52	14 58	69 10	37 94	46 24	116 41	146 87	263 3
19B	16 68	14 51	0 94	32 13	9 92	4 62	14 54	17 40	3 54	20 94	19 52	19 99‡	44 00	52 67	96 7*
20B	41 20	25 88	1 84	68 92	19 36	12 12	31 48	42 04	11 89	53 93	34 37	35 32	102 68	121 42	224 1
Average	30 77	20 68	1 36	52 81	11 27	9 58	20 85	26 52	7 34	33 86	26 72	21 65	67 99	86 85	154 8

* Averaged for check

† Sample lost

‡ 9 54, the value for 4B, taken as the check and for making up total.

The lime factor

This phase of the question has been fully discussed in an earlier paper (2); and only a brief reference need be made to it here. If the averages for the two sections (limed and unlimed) are examined, it will be noted that the corn crop of 1913 shows some increase from the lime treatment. The other crops in the rotation show little or no response to lime. The total yields for the 5 years for the limed section are only a few hundred pounds in excess of the total for the unlimed section.

These figures, taken in conjunction with nitrogen determinations made on samples of soil from both sections, indicate that there has been a greater loss of nitrogen from the limed than from the unlimed plots. Or to express the thought in another way, the acid condition of the unlimed plots has tended to conserve the nitrogen supply of the soil. It would thus appear, that for soils where oxidation of the organic matter goes on rather rapidly, the stimulating effect of lime is not required in the growing of non-leguminous crops.

TOTAL YIELD OF NITROGEN

The total yield of nitrogen for the two sections is shown in table 4.

In general, the plots that yielded the largest amounts of dry matter also yielded the largest amounts of total nitrogen. The lowest total yield for the unlimed section was 43.2 pounds per acre from 7A and the highest yield for this section was 255.3 pounds per acre from plot 18A. The lowest yield from the limed section was 79.8 pounds from plot 1B and the highest 263.3 pounds from 18B.

The average *annual* yield for all the unlimed plots was 28.3 pounds per acre and for all the limed plots 31.1 pounds per acre. The average annual yield for 18A was 51.1 pounds per acre and for 1B 52.7 pounds.

Remembering that these two plots receive between 200 and 225 pounds of nitrogen per acre annually, we see from the above figures that only about 25 per cent of the applied nitrogen is recovered in the crop.

A comparison of the total amount of nitrogen recoverd in the crops from the seven plots which receive equivalent amounts of nitrogen is of interest. These figures for both the limed and the unlimed sections are shown in table 5.

In each section the corn and wheat have drawn most heavily upon the nitrogen. The oat crop was unusually small and therefore the amount of nitrogen recovered in this crop is low. The nitrogen taken out by the timothy varies from about 23 to 30 pounds per acre annually. The corn crop in section B removed on an average 57.9 pounds of nitrogen per acre which is almost as much as is contained in 400 pounds of nitrate of soda. For the unlimed section, the seven plots which receive equivalent amounts of nitrogen show an average total of 149.6 pounds per acre of nitrogen removed by the five crops. The average total amount removed by the crops on the corresponding plots of the limed section is 170.2 pounds per acre.

Here is shown a total gain of about 20 pounds in favor of the limed plots. However, reference to the figures for the corn crop of 1913 show that the gain was practically all made by this crop.

TABLE 5

Total yields of nitrogen from plots which receive nitrogenous fertilizers in equivalent amounts

PLOT NUMBER	CORN 1913	OATS 1914	WHEAT 1915	TIMOTHY 1916	TIMOTHY 1917	TOTAL
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
9A	38.7	28.6	45.4	26.2	28.3	167.2
10A	36.9	24.6	41.0	25.6	27.8	155.9
11A	34.1	32.3	42.2	25.8	19.9	154.3
12A	35.0	26.5	39.2	23.1	22.4	146.2
13A	34.9	20.4	38.8	26.3	20.6	141.0
14A	44.6	18.6	38.6	14.0	28.3	144.1
15A	45.7	16.0	32.1	22.9	21.9	138.6
Average	38.56	23.86	39.6	23.4	24.17	149.6
9B	53.6	18.6	34.1	28.6	23.8	158.7
10B	56.5	24.9	42.4	32.9	26.0	182.7
11B	63.4	24.7	44.5	33.9	26.8	193.3
12B	56.8	21.5	38.0	34.2	21.9	172.4
13B	56.9	24.6	35.7	28.4	19.9	165.5
14B	54.8	21.2	33.5	28.2	20.0	157.7
15B	63.6	22.8	27.6	27.2	19.7	160.9
Average	57.9	22.6	36.5	30.5	22.6	170.2

The last column of figures in table 5 shows that the average amount of nitrogen recovered from the four plots (9 to 12) which receive mineral nitrogenous materials exceeds the average amount recovered from the three plots (13 to 15) which receive organic nitrogenous materials.

The 5-year averages are as follows:

	UNLIMED	LIMED
	lbs.	*lbs.*
Plots 9 to 12	155.9	176.8
Plots 13 to 15	141.2	161.3

This is in accord with the figures reported in the yield of dry matter and gives further evidence that the mineral nitrogenous materials are more efficient in crop production than the organic materials.

THE PERCENTAGE OF NITROGEN IN THE DRY MATTER

The percentage of nitrogen in the dry matter of all the crops for both the limed and the unlimed sections is shown in table 6.

For a given crop these percentages do not vary greatly, and they are therefore not of so much importance as the total nitrogen, but it seems best that they should constitute a part of the record.

TABLE 6

Percentage of nitrogen in dry matter, 1913–1917

PLOT NUMBER	CORN, 1913			OATS, 1914		WHEAT, 1915		TIMOTHY HAY		AVERAGE
	Grain	Stalks	Cobs	Grain	Straw	Grain	Straw	1916	1917	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1A	1 522	1 126	0 573	2 015	0 749	2 057	0 354	0 912	0 965	1 141
2A	1 413	0 720	0 553	1 935	0 731	2 076	0 413	1 031	1 024	1 099
3A	1 462	0 830	0 741	1 943	0 678	2 076	0 315	0 949	0 916	1 101
4A	1 364	0 781	0 405	2 015	0 597	2 017	0 384	0 939	0 945	1 049
5A	1 590	0 828	0 415	2 389	0 909	1 938	0 433	0 757	0 778	1 114
6A	1 492	0 860	0 366	2 460	1 105	2 097	0 463	0 821	0 808	1 163
7A	1 521	0 809	0 711	1 836	0 767	2 087	0 374	1 021	0 936	1 118
8A	1 373	0 611	0 435	2 149	0 785	1 780	0 345	0 730	0 729	0 993
9A	1 541	0 631	0 543	2 425	0 972	1 966	0 443	0 675	0 729	1 102
10A	1 550	1 006	0 583	2 273	0 874	1 987	0 433	0 693	0 689	1 121
11A	1 521	0 819	0 484	2 336	0 972	2 342	0 679	1 094*	1 034	1 253
12A.	1 344	0 710	0 484	2 282	0 847	2 097	0 404	0 876	0 896	1 104
13A	1 482	0 720	0 534	2 051	0 767	2 017	0 472	0 939	0 857	1 093
14A	1 680	0 740	0 484	2 238	0 838	1 849	0 462	0 857	0 778	1 103
15A	1 492	0 828	0 425	2 273	0 945	1 987	0 413	0 939	0 867	1 130
16A	1 729	1 184	0 445	2 461	0 990	2 114	0 374	0 848	0 817	1 218
17A	1 640	0 740	0 731	2 086	0 758	2 174	0 433	0 876	0 906	1 149
18A	1 690	0 917	0 395	2 452	0 998	2 263	0 473	0 857	0 995	1 227
19A	1 413	0 612	0 701	2 006	0 678	2 114	0 384	0 866	0 975	1 083
20A	1 680	0 907	0 573	2 273	0 918	2 124	0 541	0 812	0 729	1 173
Average	1 525	0 819	0 529	2 195	0 844	2 008	0 430	0 875	0 869	1 127
1B	1 600	0 898	0 464	2 122	0 811	1 978	0 384	1 040	0 945	1 138
2B	1 462	1 026	0 524	2 051	0 722	2 087	0 344	1 067	0 955	1 138
3B	1 590	0 957	0 543	2 113	0 856	2 128	0 354	1 049	0 876	1 163
4B	1 413	0 789	0 445	2 024	0 713	2 157	0 374	0 866	0 867	1 072
5B	1 620	0 947	0 415	lost	lost	2 001	0 394	0 949	0 827	1 022
6B	1 630	0 967	0 504	2 291	0 972	2 360	0 462	1 049	0 896	1 237
7B	1 600	1 016	0 415	1 792	0 660	2 069	0 482	1 012	0 867	1 101
8B	1 541	0 996	0 494	2 264	0 802	1 904	0 394	0 803	0 788	1 109
9B	1 600	0 878	0 514	2 416	1 052	2 040	0 423	0 821	0 778	1 169
10B	1 531	0 710	0 405	2 408	0 998	2 030	0 364	0 784	0 748	1 109
11B	1 482	0 878	0 445	2 389	1 016	2 080	0 453	0 830	0 758	1 148
12B	1 581	0 711	0 425	2 291	0 963	2 070	0 354	0 976	0 995	1 152
13B	1 541	0 781	0 454	2 380	0 963	2 040	0 334	0 830	0 886	1 134
14B	1 541	0 701	0 415	2 336	0 838	2 099	0 374	0 939	0 936	1 131
15B	1 571	1 045	0 385	2 398	1 052	2 021	0 334	0 894	0 896	1 177
16B	1 610	1 164	0 425	2 469	1 043	2 186	0 344	0 848	0 906	1 222
17B	1 719	1 026	0 514	2 042	0 678	2 167	0 413	0 876	1 152	1 176
18B	1 590	0 967	0 425	2 496	1 016	2 350	0 354	0 912	1 014	1 236
19B	1 334	0 631	0 534	2 068	0 678	1 933	0 305	0 976	1 162	1 069
20B	1 699	0 986	0 435	2 362	0 918	2 167	0 413	0 803	0 837	1 180
Average .	1 512	0 904	0 459	2 248	0 882	2 093	0 383	0 916	0 904	1 144

[*] A second growth—largely crab-grass—gave 0 94 per cent nitrogen.

It may be noted in passing that the corn grain contains about 1.5 per cent of nitrogen, the oats and wheat grain a little over 2 per cent and the timothy hay about 0.9 per cent. The oats straw contains about twice as much nitrogen as the wheat straw.

Attention may be called to the rather high average percentage for plots 11A, 16A, 16B, 18A and 18B. The condition of the soil on 11A is abnormal and this no doubt accounts for the high percentage of nitrogen in the crop from this plot. It has sometimes been observed that a soil very deficient in nitrogen produces a small crop having an exceptionally high nitrogen content. The other plots mentioned receive rather heavy applications of nitrogenous fertilizers, and this excess of nitrogen has influenced the nitrogen content of the crop.

It is well known, for example, that in pot experiments the normal nitrogen content of the plant may be more than doubled by heavy applications of nitrate of soda. It has also been found that the nitrogen content of leguminous plants grown on limed land is generally higher than that of the same plants grown on land that is deficient in lime.

It will be observed that there is little difference between the nitrogen content of the crops of this rotation (non-legumes) whether grown on the limed or the unlimed section.

THE PERCENTAGE OF NITROGEN RECOVERED

The check plot is supposed to show the amount of nitrogen which the crop gets from the natural supply of nitrogen that is found in the soil. Then the difference between this amount and the amount recovered through the crop receiving a nitrogenous fertilizer should show the amount of the applied nitrogen which that crop was able to win back, or recover. From this figure the percentage of applied nitrogen may be calculated.

The percentage recovery for the various nitrogenous materials has been thus calculated for the 5-year period and is shown in table 7.

Attention may be drawn to the low average recovery from plots 5, 6 and 18. All these plots receive annual applications of manure at the rate of 16 tons per acre and in addition plot 18 receives 320 pounds of nitrate of soda per acre. Less than one-sixth of the applied nitrogen is won back in the crops. There is, therefore, a heavy loss of nitrogen from these plots. No doubt much of this loss is due to the escaping of nitrogen gas and ammonia while decomposition is taking place. So far, no direct means has been found of measuring such losses.

Analysis shows that these soils have made slight gains in nitrogen content since the work was started in 1908. But as Russell (3) points out, such gains cannot go on indefinitely. There comes a time, he says, when "a point of equilibrium is reached, higher or lower according to the soil conditions, where further gains are balanced by losses, so that the nitrogen content remains constant." It would appear from the heavy loss sustained by these plots that the point of equilibrium has been reached and that the gains are balanced by the losses. The fact that these plots are receiving excessive amounts of nitrogen is attested by the serious lodging of the grain.

TABLE 7

Percentage of nitrogen recovered in the different crops

PLOT NUMBER	CORN, 1913	OATS, 1914	WHEAT, 1915	TIMOTHY HAY 1916	TIMOTHY HAY 1917	5-YEAR AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
5A	20.38	7.98	18.25	11.22	15.24	14.61
6A	17.72	7.54	17.53	18.77	19.25	16.16
8A	Loss	15.72	15.87	20.91	29.22	16.34
9A	34.39	19.62	48.34	25.77	42.07	34.04
10A	30.63	11.52	39.58	24.65	41.14	29.50
11A	25.01	26.88	42.00	24.91	24.61	28.68
12A	26.80	15.31	35.97	19.52	29.88	25.50
13A	26.53	3.15	35.30	25.98	26.09	23.41
14A	46.39	Loss	34.88	22.00	42.14	29.08
15A	48.61	Loss	21.87	19.07	28.75	23.66
16A	28.70	4.57	19.70	14.41	20.79	17.63
17A	12.45	Loss	2.71	35.50	13.60	12.85
18A	22.94	5.05	25.19	11.70	14.87	15.95
20A	17.07	11.02	35.74	30.25	27.30	24.28
Average	25.54	9.17	28.07	21.76	26.78	22.26
5B	15.64	Sample lost	14.55	8.67	12.49	12.84*
6B	4.11	5.02	17.49	13.05	14.79	10.89
8B	71.98	42.28	33.66	21.81	25.74	39.09
9B	35.97	11.16	30.73	22.87	29.53	26.05
10B	41.88	23.81	47.44	31.57	34.12	35.76
11B	55.94	23.41	51.59	33.47	35.78	40.04
12B	42.55	16.91	38.57	34.09	25.56	31.53
13B	42.61	23.49	34.02	22.30	21.33	28.75
14B	38.38	16.31	29.57	21.85	21.71	25.56
15B	56.41	19.46	17.81	19.89	21.05	26.92
16B	20.99	8.66	18.58	10.01	19.33	15.51
17B	39.69	13.67	34.11	39.65	68.70	39.16
18B	17.14	10.45	24.91	9.81	16.04	15.67
20B	23.36	28.21	41.59	24.51	28.84	29.30
Average	36.19	18.68	31.04	22.40	26.80	26.93

* 12.84 is 4-year average.

Note—Plots 1, 2, 3, 4, 7, and 19 received no nitrogen.

It is of interest to compare the percentage of nitrogen recovered from the four mineral nitrogenous plots with the recovery from the three plots which receive organic materials. The averages for these plots for the two 5-year periods and for the limed and the unlimed section are shown in table 8.

Throughout the 10 years and for both sections the mineral materials have given the highest percentage recovery.

The differences are not so great, however, for the second 5-year period as for the first. This may mean some cumulative effect from the organic materials, but in the cylinder experiments referred to such cumulative effect, if it exists, has not brought the recovery from an organic material up to the level of the mineral materials during a period of 20 years.

It may be pointed out that during the second 5-year period only plots 10B and 11B of the seven under consideration show a recovery of more than one-third of the applied nitrogen.

It is exceedingly unfortunate that so much of this most expensive element must be lost. The situation should stimulate soil investigators everywhere to put forth efforts to find a means of stopping some of this loss. Furthermore it should be a warning to the farmer to secure a greater proportion of his nitrogen from the atmosphere through means of leguminous crops. If he must lose two-thirds or more of his purchased nitrogen then it will be economy to purchase only such amounts as can be used most effectively.

TABLE 8

Per cent of nitrogen recovered from mineral nitrogenous materials as compared with organic materials

	UNLIMED SECTION		LIMED SECTION	
	1908–1912	1913–1917	1908–1912	1913–1917
	per cent	*per cent*	*per cent*	*per cent*
Average, 4 mineral materials.	40 1	29 4	48 5	33 3
Average, 3 organic materials	27 3	23 5	28.5	27 1

Indeed it has been shown that the percentage recovery is generally higher with small than with large applications. Thus small applications supplemented by the nitrogen secured through legumes will enable the farmer to get better results and with less cash outlay.

An examination of the table shows that less nitrogen has been recovered from 5B, 6B and 18B than from the corresponding A plots. This means that on these plots available nitrogen is not the limiting factor. Here nitrification takes place rapidly enough without the stimulating effect of carbonate of lime. Lime simply adds fuel to the fires of destruction.

Plot 8A gives a 5-year average return of 16.34 per cent, whereas 8B, the corresponding limed plot, shows a recovery of 39.09 per cent. This emphasizes the value of a small amount of available nitrogen when lime is supplied. On the unlimed section nitrate of soda (plot 9A) shows a higher average recovery than either ammonium sulfate, calcium nitrate or calcium cyanamid, but on the limed section all these show a higher recovery than the nitrate.

A 5-year average recovery of 40 per cent for 11B (the highest 5-year average) gives evidence of the value of lime in promoting nitrification in a soil that would otherwise have been made acid by the continued use of sulfate of ammonia. In contrast 11A which is the unlimed sulfate of ammonia plot, shows a recovery of 28.68 per cent.

Plot 17B, which receives its nitrogen in the form of rye straw (a slowly available material) and is limed, shows a recovery of 39.16 per cent as against a recovery of 12.85 per cent for 17A, the corresponding unlimed plot.

The seven unlimed plots (9A to 15A) which receive equivalent amounts of nitrogen, show a 5-year average recovery of 27.7 per cent as against 30.7 per cent for the corresponding limed plots, a difference of 3 per cent in favor of the latter.

THE INCOME AND OUTGO OF NITROGEN

This question has been partially covered by the discussion under "Percentage of Nitrogen Recovered." It seems worth while, however, to record here the amount of nitrogen applied each year and the total amount recovered in the crops for the 5 years. These figures, on the acre basis, are shown in table 9.

It may be noted that plots 5, 6 and 18 receive much larger applications of nitrogen than plots 9 to 15, inclusive. Plot 18 received during the 5 years nitrogen equivalent to 1081.6 pounds per acre, which is more than four times the standard application. The percentage of nitrogen recovered from this plot has already been discussed.

The variations in the amount of nitrogen furnished by the rye-straw, plot 17, are probably due to the fact that in some cases more grain was left in the straw than in others.

Through an oversight the alfalfa hay and rye straw used on plots 16 and 17 in 1916 were not analyzed. It was therefore necessary to estimate the amount of nitrogen in these materials.

The percentage of the applied nitrogen that was recovered in the crop is shown in the last column of the table. These figures should correspond with the figures given in table 7, but on account of slight differences in decimals and irregularities in two or three of the series, there are some variations.

A study of the income and outgo of nitrogen would not be complete without a consideration of the nitrogen content of the soil. Unfortunately samples from only a few of the plots were analyzed when the work was started; however, these analyses may be taken as a fair average of the nitrogen content of the soil at that time. Analyses made in 1909 of samples from five of the plots show an average nitrogen content of 0.1118 per cent and a carbon content of 1.22 per cent. Samples were collected from all the plots at the end of the first 5-year period and again at the end of the second 5-year period (1917), and nitrogen and carbon determinations have been made on all these samples, the results of which are reported in table 10.

TABLE 9

Nitrogen applied for each crop (acre basis) and the average percentage of nitrogen recovered

	NITROGEN APPLIED, 1913	NITROGEN APPLIED, 1914	NITROGEN APPLIED, 1915	NITROGEN APPLIED, 1916	NITROGEN APPLIED, 1917	TOTAL NITROGEN APPLIED	TOTAL NITROGEN RECOVERED IN CROPS	TOTAL INCREASE OVER CHECK	AVERAGE PERCENTAGE OF NITROGEN RECOVERED
	lbs.	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*per cent*
1A..........							101 7		
2A							94 9		
3A							96 9		
4A							90 5	Check	
5A	173 5	169 0	152 3	159 7	180 5	835 0	205 4	122 0	14 6
6A	215 8	227 2	203 5	129 7	147 5	923 7	227 2	143 8	15 6
7A							43 2		
8A	24 5	25 0	25 0	24 5	24 1	123 1	103 3	19 9	16 2
9A	49 0	50 1	50 1	49 0	48 3	246 5	167 2	83 8	34 0
10A	49 0	50 1	50 1	49 0	48 3	246 5	156 0	72 6	29 5
11A	49 0	50 1	50 1	49 0	48 3	246 5	154 1	70 7	28 7
12A	49 0	50 1	50 1	49 0	48 3	246 5	146 2	62 8	25 5
13A	49 0	50 1	50 1	49 0	48 3	246 5	141 0	57 6	23 4
14A	49 0	50 1	50 1	49 0	48 3	246 5	154 5	71 1	28 9
15A	49 0	50 1	50 1	49 0	48 3	246 5	138 6	55 2	22 4
16A	147 3	100 2	102 0	100 0*	96 9	546 4	184 8	101 4	18 6
17A	92 1	15 4	34 7	20 0*	40 1	202 3	104 0	20 6	10 2
18A	222 5	219 1	202 5	208 6	228 9	1081 6	255 3	171 9	15 9
19A							76 2	Check	
20A	141 1	65 5	84 8	69 0	89 4	449 8	200 2	116 8	26 0
1B							79 9		
2B							94 5		
3B							92 9		
4B							92 6	Check	
5B	173 5	169 0	152 3	159 7	180 5	835 0	167 3	72 7	8 70
6B	215 8	221 8	203 5	129 7	147 5	918 3	189 0	94 4	10 28
7B							100 3	5 7	
8B	24 5	25 0	25 0	24 5	24 1	123 1	142 9	48 3	39 2
9B	49 0	50 1	50 1	49 0	48 3	246 5	158 6	64 0	26 0
10B	49 0	50 1	50 1	49 0	48 3	246 5	182 8	88 2	35 8
11B	49 0	50 1	50 1	49 0	48 3	246 5	193 4	98 8	40 1
12B	49 0	50 1	50 1	49 0	48 3	246 5	172 3	77 7	31 5
13B	49 0	50 1	50 1	49 0	48 3	246 5	165 4	70 8	28 7
14B	49 0	50 1	50 1	49 0	48 3	246 5	157 6	63 0	25 6
15B	49 0	50 1	50 1	49 0	48 3	246 5	160 8	66 2	26 9
16B	147 3	100 2	102 0	100 0*	96 9	546 4	181 9	87 3	16 0
17B	92 1	15 4	34 7	20 0*	40 1	202 3	180 6	86 0	42 5
18B	222 5	219 1	202 5	208 6	228 9	1081 6	263 3	168 7	15 6
19B							96 67	Check	
20B	141 1	65 5	84 8	69 0	89 4	449 8	224 1	129 5	28 8

* Estimated; straw and alfalfa hay not analyzed.

TABLE 10

Percentage of total nitrogen and carbon in the unlimed and the limed soils in 1913 and 1917

PLOT NUMBER	FERTILIZER TREATMENT FOR $\frac{1}{20}$ ACRE PLOT	UNLIMED SECTION (A PLOTS)				LIMED SECTION (B PLOTS)			
		Nitrogen		Total carbon		Nitrogen		Total carbon	
		1913	1917	1913	1917	1913	1917	1913	1917
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	Nothing ..	0 1059	0 1033	1 39	1 33	0 0862	0 0770	1 06	0 90
2	16 pounds muriate of potash	0 1098	0 1173	1 44	1 33	0 0871	0 0790	1 14	0 96
3	32 pounds acid phosphate	0 1042	0 1139	1 28	1 24	0 0821	0 0751	1 07	0 86
4	*Minerals only	0 1180	0 1088	1 36	1 26	0 0855	0 0779	1 15	1 04
5	Minerals, 1600 pounds cow manure	0 1002	0 1185	1 38	1 44	0 1030	0 1143	1 44	1.42
6	Minerals, 1600 pounds horse manure	0 0929	0 1230	1 39	1 57	0 1028	0 1190	1 43	1 48
7	Nothing	0 0790	0 0785	0 95	0 93	0 0879	0 0821	1 17	1 02
8	Minerals, 8 pounds $NaNO_3$.	0 0880	0 0883	1 11	1 03	0 0801	0 0787	1 08	1 01
9	Minerals, 16 pounds $NaNO_3$.	0 0921	0 0975	1 15	1 13	0 0753	0 0810	1 08	0 96
10	Minerals, $Ca(NO_3)_2$ equivalent to 16 pounds $NaNO_3$	0 0941	0 0903	1 14	1 10	0 0805	0 0779	1 12	1 01
11	Minerals, $(NH_4)_2SO_4$ equivalent to 16 pounds $NaNO_3$	0 0977	0 0904	1 14	1 21	0 0801	0 0819	1 02	1 06
12	Minerals, $CaCN_2$ equivalent to 16 pounds $NaNO_3$	0 0869	0 0844	1 03	1 02	0 0861	0 0896	1 16	1 07
13	Minerals, dried blood equivalent to 16 pounds $NaNO_3$	0 0925	0 1063	1 14	1 23	0 0807	0 0826	1 05	0 98
14	Minerals, fish equivalent to 16 pounds $NaNO_3$	0 1014	0 1058	1 26	1 22	0 0779	0 0889	1 08	0 93
15	Minerals, concentrated tankage equivalent to 16 pounds $NaNO_3$	0 0884	0 0919	1 21	1 18	0 0717	0 0803	0 93	0 89
16	Minerals, 200 pounds alfalfa hay	0 0891	0 1012	1 20	1 14	0 0897	0 0904	1 18	1 10
17	Minerals, 200 pounds wheat or rye straw	0 0950	0 0980	1 26	1.08	0 0953	0 0952	1 25	1 15
18	Minerals, 1600 pounds cow manure and 16 pounds $NaNO_3$	0 1088	0 1170	1 47	1.36	0 1014	0 1190	1 41	1 48
19	Minerals only	0 0980	0 0872	1 29	1 01	0 0809	0 0784	1 11	0 95
20	Minerals, 200 pounds wheat or rye straw and 16 pounds $NaNO_3$	0 0959	0 0908	1 23	1 03	0 0832	0 0903	1 14	1 12
Average		0 0969	0 1006	1 24	1 19	0 0859	0 0879	1 15	1 07

* Minerals—32 pounds acid phosphate and 16 pounds muriate of potash.

An examination of the average nitrogen content for all the unlimed plots shows that there is now approximately 0.01 per cent, or 200 pounds per plowed acre less nitrogen in these soils than there was in 1909. The corresponding loss from the limed section amounts to nearly 500 pounds of nitrogen per acre. Thus the limed plots have lost about 250 pounds more of nitrogen per acre than the unlimed plots. The probable reason for this greater loss from the limed than from the unlimed section has been discussed in an earlier paper (2) and only brief reference need be made to it here. It would appear in the case of the unlimed plots that the acidity of the soil has acted as a check on the breaking up of organic matter and therefore on the loss of nitrogen. On the other hand the well limed plots furnished a favorable medium for those organisms that destroy organic matter and as a consequence the loss of nitrogen was heavy. We have here a verification of the popular view that lime tends to "burn out" the organic matter; only the "burning out" must be recognized as an indirect rather than a direct result of the lime. This rather rapid oxidation of organic matter results in the formation of available nitrogen compounds which should be utilized by the growing crops.

In this case, however, it will be remembered that there was very little more dry matter produced on the limed than on the unlimed plots. On these plots nitrogen was *not* the limiting factor and the extra amount of available nitrogen compounds was largely lost. From this we may conclude that on soils that are not especially heavy, and with non-legume crops, oxidation may proceed rapidly enough without the stimulating effect of lime compounds.

It is interesting to note that during the period 1913–1917 there was little change in the average nitrogen content of soil in the two sections. Such change as there was is in the direction of a gain. It is possible that in 1913 the equilibrium of which Russell speaks had been reached and from this time on the gains equaled the losses. If this explanation is accepted it must be admitted that the equilibrium point was lower for the limed than for the unlimed section.

It is of especial interest to note that the nitrogen content of those plots which have received manure and manure with nitrate of soda has been maintained just about on a level with the nitrogen content of the original soil.

The high nitrogen and carbon content of plots 1A to 4A which during the 10 years have received no nitrogen tends to confirm the suggestion which has already been made, viz.: that the soil was naturally better here than in the remaining part of the section. A nitrogen content of 0.079 per cent for 7A illustrates well what will happen when land is cropped continuously without fertilizers or manure. This plot shows a greater loss of nitrogen than any of the others in this section. Assuming that it originally contained 0.1118 per cent, which represents the average of the plots which were sampled in 1909, it has lost nitrogen at the rate of about 600 pounds to the plowed acre.

In most cases, as has already been pointed out, the limed plots have lost more nitrogen than the corresponding unlimed plots, but it happens that this is not true of 7B which is the limed unfertilized plot.

The average percentage of carbon in samples from the unlimed plots is practically the same in 1913 as in the orginal soil, but the limed section shows a decrease during this period of about 0.07 per cent, or 1400 pounds to the plowed acre. This only emphasizes what has been said with regard to the more rapid oxidation of organic matter on the limed plots.

It will be noted also that the average per cent of carbon is less for both sections in 1917 than it was in 1913. On the other hand, plots 5, 6 and 18 in each section show a decided increase in percentage of carbon over the original soil, the carbon content of which was 1.22 per cent. These plots receive an annual application of manure at the rate of 16 tons per acre and an increase in carbon content is to be expected. The large amount of nitrogen that is supplied causes a rank growth of grain and grass and thus there is left on these plots a heavier stubble or sod than on the plots that receive less nitrogen. These residues supplement the organic matter which is supplied by the manure.

There is quite a contrast between 6A and 7A which lie side by side. In 1917 the former contained 1.57 per cent of carbon and the latter (the no-fertilizer plot) 0.93 per cent. Even on the limed section the nitrogen content of 6A has been increased.

It is of interest to compare the average nitrogen and carbon in the soil from the seven plots (9 to 15) which receive equal amounts of nitrogen with the average for all the plots. The averages are as follows:

TREATMENT	NITROGEN		CARBON	
	1913	1917	1913	1917
	per cent	*per cent*	*per cent*	*per cent*
Plots 9 to 15, unlimed	0.0933	0.0952	1.15	1.16
General average, unlimed	0.0969	0.1006	1.24	1.19
Plots 9 to 15, limed	0.0789	0.0832	1.06	0.99
General average, limed	0.0859	0.0876	1.15	1.07

Here, as in the general average, there is less nitrogen and carbon in the soil of the limed than of the unlimed section. In all cases, too, the average for these seven plots is less than the general average for the twenty plots.

From this it is evident that the heavy applications of manure have aided very materially in bringing up the general average.

SUMMARY

This is a report for the second 5-year period of work that was started in 1908, the object of which is a study of the relative availability of different nitrogenous materials, and of nitrogen losses under a 5-year rotation of corn, oats, wheat and two years of timothy.

In laying out the experiment forty 1/20-acre plots were provided in parallel sections of twenty plots each, so that the nitrogen work could be duplicated, the one section designated as "A" remaining unlimed and the other designated as "B" to be limed at stated intervals.

Both sections have been supplied with liberal amounts of phosphorus and potassium so that these might not become limiting factors.

With slight exceptions the mineral nitrogenous materials have increased the yields over the yields from the check plots.

The average yields of dry matter, and the percentage of nitrogen recovered have been greater with the mineral nitrogenous materials than with organic materials.

Of the four mineral materials, nitrate of soda gave the largest yields of dry matter, and the highest percentage of nitrogen recovered on the unlimed section, and sulfate of ammonia the highest on the limed section.

Of the three commercial organic materials, dried fish gave the highest yields of dry matter and the highest percentage of nitrogen recovered on the unlimed section, and dried blood the highest on the limed section.

Farm manure, and farm manure with nitrate of soda gave the largest total yields, but on account of the large excess of nitrogen supplied by these materials the increases can hardly be considered profitable when compared with the increase made by the commercial nitrogenous materials.

The four mineral nitrogenous materials gave for the period 1913–1917 an average recovery of 29.4 per cent of nitrogen for the unlimed section and 33.3 per cent for the limed section. The corresponding figures for the three organic materials are 23.5 per cent and 27.1 per cent. There is thus shown an average loss of more than two-thirds of the applied nitrogen.

With the exception of the corn crop of 1913 the yields on the limed section were about the same as on the unlimed section.

There is practically no difference in percentage of nitrogen in the dry matter from the limed and the unlimed sections.

Analyses of samples of soil from plots of the two sections show that the limed plots have lost, during the 10 years, nearly 250 pounds more of nitrogen than the unlimed plots. The loss of carbon also has been greater on the limed than on the unlimed section.

It is suggested that in the case of soils of this type and with no legume crops in the rotation to furnish additional organic matter, oxidation may go on rapidly enough without the stimulating effect of lime.

The supply of nitrogen and carbon has been best maintained on those plots which have received the farm manure and the farm manure plus nitrate of soda. The carbon content of these plots has even been increased over the carbon content of the original soil.

The work emphasizes the difficulty of maintaining the nitrogen supply of the soil at a high level under continuous cropping to *non-leguminous* crops, even when *commerical fertilizers* are supplied in generous amounts.

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THE RELATION OF SULFUR TO SOIL ACIDITY AND TO THE CONTROL OF POTATO SCAB[1]

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INTRODUCTION

Potato scab, caused by *Actinomyces chromogenus* Gasperini, is no doubt, the most common disease of this important agricultural crop. The disease was first mentioned in Loudon's Encyclopedia of Agriculture (18) as follows: "Scab, that is to say, the ulceration of the surface of the tubers, has never been explained in a satisfactory manner. Some attribute it to the ammonia from the dung of the horse, others to alkali, and certain others to the use of wood ashes on the soil. Not using diseased seed and planting on other soil are the only known means of preventing the malady." Since this first mention of the disease an extensive literature has appeared on the subject, a large portion of which has been devoted to control measures.

On the tuber, satisfactory control has been secured by treating with either formaldehyde or corrosive sublimate. Where the scab organism, is present in the soil, however, as is very frequently the case where potatoes are grown for a number of years on the same land, seed treatment is of little value. Where this condition exists, control measures must be based on eradicating the organism from the soil. This fact has long been recognized, and Halsted (10, 11, 12, 13, 14), one of the first to conduct investigations on this subject, found from his tests with various soil germicides that sulfur was the only one which gave results that would warrant its use for this purpose.

Further investigations by Halsted and others, have shown contradictory results in the use of sulfur for the control of scab. Halsted (10, 11, 12, 13, 14) reports a marked decrease in the amount of scab following an application of 300 pounds of sulfur per acre, while a similar quantity under different conditions had no beneficial effect. In another experiment, an application of 600 pounds of sulfur in 1896 had no effect on scab in that or the succeeding year; in 1898 however, the sulfur became effective, bringing about a reduction of 36.1 per cent in the amount of scab.

In an experiment conducted in the greenhouse, Garman (6) found no difference in the amount of scab where untreated seed pieces, and seed pieces rolled in sulfur flour, were planted in sterile soil. Field experiments where sulfur was used at the rate of 150 and 180 pounds per acre likewise showed no indicative results. His results were corroborated by Brooks (3) who reports failure in the use of sulfur for the control of scab.

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[1] Technical Paper No. 3 of the New Jersey Agricultural Experiment Stations Department of Plant Pathology.

As a result of a series of experiments, in which sulfur was used at the rate of 300 pounds per acre following an application of 600 pounds in the preceding year, Wheeler and Adams (22) conclude that: "The indications are that the sulfur treatment of contaminated soils may decidedly reduce the percentage of scab if enough sulfur is employed and the moisture and other soil conditions are such that it is able to exert its maximum effect."

The rôle that sulfur plays in the soil is an undecided question. Chancrin and Desriot (4) express the opinion that its beneficial effects may be due to action similar to that of partial sterilization by heat, carbon-bisulfide, toluene, etc. In its use for the control of scab its beneficial action would appear to be due to the fact that on its oxidation there is an increase in soil acidity, thus producing conditions unfavorable for the development of the scab organism. Numerous investigators, among whom might be mentioned Brioux and Guerbet (1), Brown and Kellogg (2) and Lipman and his co-workers (17) have demonstrated that the presence of sulfofying organisms as well as the moisture relations are factors of considerable importance in the oxidation of sulfur. Since it is a well established fact that the biological factor is influential in the process it is possible that some of the reported failures of sulfur applications to control scab may be explained on the ground that the sulfur was not oxidized.

In a series of carefully performed field experiments, Sherbakoff (19) applied sulfur at the rate of 450 and 900 pounds per acre. In every case there was a reduction in the amount of scab, the heavier application giving the greatest decrease. Included in these experiments were tests to determine the influence of the time of application of the sulfur. Most efficient control was secured where the sulfur was broadcasted just before the potatoes were planted. Mixing the sulfur with the fertilizer not only reduced the fungicidal effect of the sulfur but reduced the value of the fertilizer as well. In another paper Sherbakoff (20) reports that where heavy applications of sulfur were made injurious effects were apparent on clover the following year, the injury being in proportion to the amount of sulfur applied.

Lint (16) conducted field experiments with sulfur over a period of three years and found that any quantity over 600 pounds is not to be advised except under especially alkaline conditions. In cases where sulfur was applied at the rate of 600 and 800 pounds per acre in the row, Lint reports injury on the succeeding hay crop. Where smaller amounts were used, and the applications made broadcast the danger of injury was reduced to a minimum.

From the brief review of the previous investigations on the use of sulfur for the control of potato scab, it is apparent that the chief difficulty lies in determining the amount necessary to change the soil reaction to a degree that will inhibit the growth of the scab organism without interfering with the proper development of the potato crop or of subsequent crops. That the soil acidity may be increased to this extent without injury to the potato crop, is evident from the work of Gillespie and Hurst (8). They have shown that while the Caribou loam soils of Maine are more acid than the Washburn loam soils, the potatoes grown on the former are not only free from scab but give larger yields.

In a recent paper having an important bearing on the question of the eradication of the scab organism from the soil, Gillespie and Hurst (9) show that an excellent correlation exists between the hydrogen-ion concentration and the occurrence of the potato scab organism. They examined a number of potato soils of different origin and type and found that those having a hydrogen-ion concentration as low as 5.2 rarely produced scabby tubers while potatoes grown on soils having higher exponents generally were scabby. Among the samples examined by them were two from the same field, a portion of which was limed in 1906 while the remainder received no lime. The limed portions produced scabby potatoes in 1906 and again in 1917, while the potatoes from the unlimed portion were clean both years. On determining the hydrogen-ion concentration of soil samples from the two sections, it was found that the limed and unlimed portions gave pH values of 5.7 and 5.05, respectively. In another paper Gillespie (7) reports the results of studies made of the viability of a number of strains of the scab organism in culture media adjusted to various hydrogen-ion exponents. He found that in a medium having a pH value of 5.2 growth was slower and generally less vigorous than it was in media having higher pH values. In some instances strains of the organism succeeded in growing well in a medium which had an initial pH value of 4.8 but the growth was accompanied by a decrease in acidity. The writer states that it is doubtful if more than a poor growth can occur at such exponents.

While the results of previous experiments with the use of elemental sulfur for the control of potato scab have been contradictory, the evidence as a whole indicates the possibility of its use in this connection. In view of the fact that potato scab is becoming more and more destructive, it was thought advisable that further research be conducted on this problem. Since the presence or absence of the scab organisms appears to be determined to a large extent by the soil reaction, in the experiments here reported particular attention was given to the relation of the organism to soil acidity as measured by the hydrogen-ion concentration and the relation of the latter to the amount of sulfur applied to the soil.

EXPERIMENTAL

During the summer of 1919, five field experiments were conducted. These experiments were performed on different soil types including sandy loam, Sassafras loam and Penn loam. In selecting the fields in which the experiments were conducted, care was taken to avoid any marked soil irregularities so that differences arising from soil variations might be reduced to a minimum. As an additional precaution check plots were left between each two treated plots and each treatment was repeated at least three times. The size of the plots in the different experiments varied from $\frac{1}{40}$ to $\frac{1}{80}$ acre. Except for the sulfur treatments all the plots of an experiment were treated alike as regards fertilization and cultivation.

Sulfur was used in amounts varying from 300 to 1200 pounds per acre. The sulfur used was the commercial flour sulfur. The applications were made broadcast after the land was harrowed and just before planting. In one of the experiments the sulfur was applied by hand, in two others a lime distributor was employed while in the remaining two the applications were made with a grain drill. The latter method proved the most efficient since the sulfur was thoroughly mixed with the surface soil and a more uniform distribution was secured.

Experiments were conducted with the varieties American Giant and Irish Cobbler. When the potatoes were harvested they were separated into two classes, primes and seconds, the latter including all tubers under 1½ inches in diameter. The primes were then divided into classes depending on the degree of infection. In grading the primes of the American Giant variety, two classes were made, clean and scabby, the latter including all tubers showing any scab lesions. Three classes were made of the Irish Cobbler primes, namely, clean, salable scabby and unsalable scabby. The last class was made up of all tubers covered with the scab lesions while tubers showing only a moderate infection were designated as salable scabby. In this connection, it must be stated that the percentage of salable scabby tubers in the primes is not a fair index of the actual control of scab since the salable scabby tubers from the check plots showed considerably more scab than those from the treated plots. This is particularly true of the American Giant variety, the primes in the sulfur-treated plots not only showed less scab than those from the check plots but were of a much better color and texture.

Before making the sulfur applications, soil samples were taken in the area to be included in the experiment and the hydrogen-ion concentration of water extracts of the soil samples determined colorometrically, following the work by Clark and Lubs (5) in the preparation of the buffer mixtures, the selection of suitable indicators and general methods of procedure. When the crop was harvested, soil samples were taken in each plot and similar determinations were made. In taking the soil samples borings were made to a depth of 6⅔ inches, at intervals of 15 feet. These individual samples were then thoroughly mixed and a sample taken to represent the condition of the plot in question.

In preparing the water extracts of the soil samples to be tested a method was adopted which was essentially the same as that employed by Gillespie and Hurst (9). To 15 gm. of air-dry soil which had been passed through a 1-mm. sieve was added 30 cc. of distilled water in a 100-cc. Erlenmyer flask. The flask was then shaken 75 times, and allowed to stand for a period of 8 to 12 hours. The supernatant liquid was then drawn off and distributed to test tubes.

The yields per acre as well as the percentage of scabby tubers will be presented in the following tables in which the data given are averages obtained from at least three replications of each treatment and of six check plots. The yields of second-size tubers are included in the tables; however, in view of the fact that the sulfur treatments appeared to have no influence on the number of seconds, they will not be discussed.

Experiments with the Irish Cobbler Variety

Experiment I. The soil on which this experiment was conducted is a sandy loam, typical of one of the best potato-growing sections of New Jersey. In 1912 an application of lime was made at the rate of 1200 pounds per acre; since that time succeeding potato crops have been severely scabbed, a large portion of the crop being unsalable in 1919. Soil samples taken before the sulfur applications were made showed a hydrogen-ion exponent of 6.15. In this experiment sulfur was used at the rate of 400 and 600 pounds per acre. Observations made at frequent intervals during the summer on this and the other experiments conducted failed to show any differences in vegetative growth that could be attributed to the sulfur applications.

TABLE 1

Influence of sulfur applications on total yield, per cent of scabby tubers and hydrogen-ion concentration

TREATMENT	TOTAL YIELD	YIELD OF PRIMES			YIELD OF SECONDS	pH VALUE OF SOIL EXTRACTS
		Salable		Unsalable, scabby		
		Clean	Scabby			
	bushels per acre	*bushels per acre*	*per cent*	*bushels per acre*	*bushels per acre*	
Check*	350.1	163.5	64.6	146.8	39.7	6.03
400 pounds sulfur per acre†	339.1	265.8	29.4	30.2	42.6	5.20
600 pounds sulfur per acre†	342.7	283.2	19.4	25.9	33.4	5.07

* Average of 6 plots.

† Average of 4 plots.

The effects of the sulfur on total yield and scab control are shown in table 1. It will be seen that there was a slight decrease in yield on the treated plots as compared with the check plots; it is doubtful, however, if these differences can be attributed to the action of the sulfur, since the decrease was greater for the 400 than for the 600-pound application. With both quantities of sulfur used, there was a marked gain in the number of salable primes with a corresponding decrease in the number of tubers rendered unsalable by scab. In addition to this the per cent of scabby tubers in the salable primes was greatly reduced by the sulfur applications.

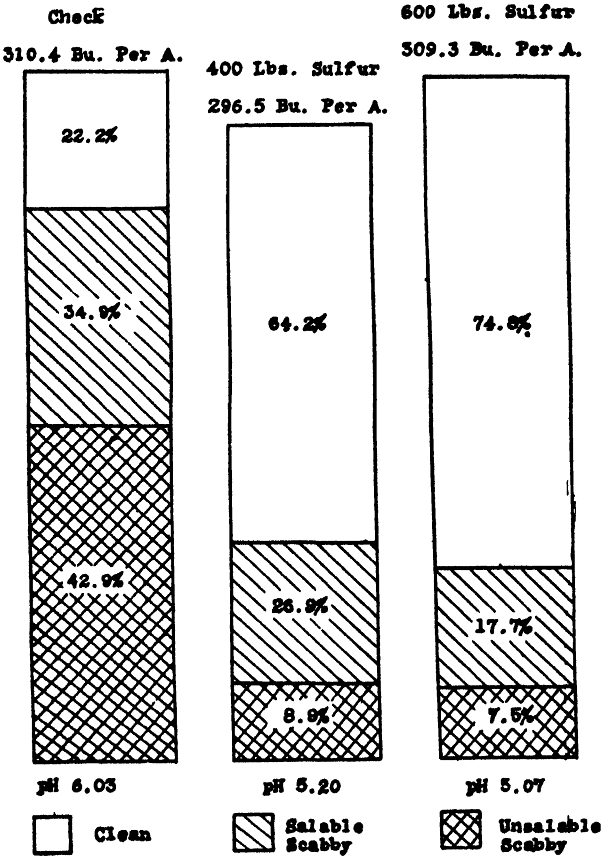

FIG. 1. DIAGRAM SHOWING THE RELATION OF SULFUR TREATMENT TO HYDROGEN-ION CONCENTRATION AND THE PER CENT OF CLEAN, SALABLE AND UNSALABLE SCABBY TUBERS IN THE PRIMES—IRISH COBBLER VARIETY, EXPERIMENT I

The relation of the hydrogen-ion exponent to the per cent of clean, salable scabby and unsalable scabby tubers in the primes is shown in figure 1. The exponent of the check plots when harvested was found to be 6.03; as has been stated, the original exponent for this soil was 6.15, indicating that little change occurred during the growing season. On the other hand, the plots receiving the sulfur applications showed a decided decrease in exponents, the decrease being more pronounced on the plots receiving the heavier applications. From the diagram it is apparent that with the decrease in the hydrogen-ion exponent there was a corresponding decrease in the per cent of scabby tubers as compared with the check plots. It will be observed, however, that even where the larger quantities of sulfur were used the crop was not entirely free from scab, this despite the fact that the exponent of these plots was 5.07, slightly lower than that shown by Gillespie (7) to delay or inhibit the growth of the scab organism in culture media. This would indicate either that there was not a uniform distribution of the sulfur with the consequence that there was no resulting acidity in those areas not receiving sulfur, or that the organism will grow at lower exponents in soil than in culture media.

Experiment II. The soil on which this experiment was conducted is a sandy loam of a heavier type than that on which the first experiment was performed. Scab has been very severe in this field for a number of years. Sulfur was applied at the rate of 300 and 600 pounds per acre.

TABLE 2

Influence of sulfur on total yield, per cent of scabby tubers and hydrogen-ion concentration

TREATMENT	TOTAL YIELD	YIELD OF PRIMES			YIELD OF SECONDS	pH VALUES OF SOIL EXTRACTS
		Salable		Unsalable scabby		
		Clean	Scabby			
	bushels per acre	*bushels per acre*	*per cent*	*bushels per acre*	*bushels per acre*	
Check*. . . .	174.0	70.4	57.6	85.9	17.7	5.57
300 pounds sulfur per acre† .	181.4	144.6	22.4	24.1	12.7	4.77
600 pounds sulfur per acre†	171.9	132.0	23.8	22.9	17.1	4.82

* Average of 6 plots.

† Average of 4 plots.

The results of the sulfur treatments are given in table 2. On a basis of total yield, the plots receiving 300 pounds of sulfur showed an increase as compared with the untreated plots, while the plots treated with 600 pounds of sulfur per acre showed a slight decrease. The differences in either case are small, however, and it is doubtful whether any importance can be attached to them. As in the preceding experiment, there was a marked reduction of unsalable scabby tubers on the treated plots leading to an increase in the number of salable primes. The per cent of scabby tubers in the salable primes was likewise greatly reduced.

In figure 2 is shown the relation of the hydrogen-ion exponent to the per cent of salable and unsalable scabby tubers in the primes. The initial hydrogen-ion exponent of this soil was 5.6 as compared with 5.57 for the check plots at the time of harvesting. It will be seen from the diagram, that the exponent of the plots treated with 300 pounds of sulfur is slightly lower than that of the plots receiving double this amount. This indicates that the sulfur had not all been oxidized at the time of digging. This is corroborated by the work of Shedd (21) who has shown that after about four months the amount of sulfur oxidized was generally about 60 per cent of the total quantity present regardless of whether 100 or 500 pounds per acre had been added. It will further be seen from the diagram, that scab was approximately as well controlled on the plots receiving 300 pounds of sulfur per acre as it was on the plots where 600 pounds were used. It thus appears from the results of this experiment that, for this particular soil type, having the hydrogen-ion exponent as low as 5.6, the use of sulfur in any quantity exceeding 300 pounds was unnecessary for the control of scab.

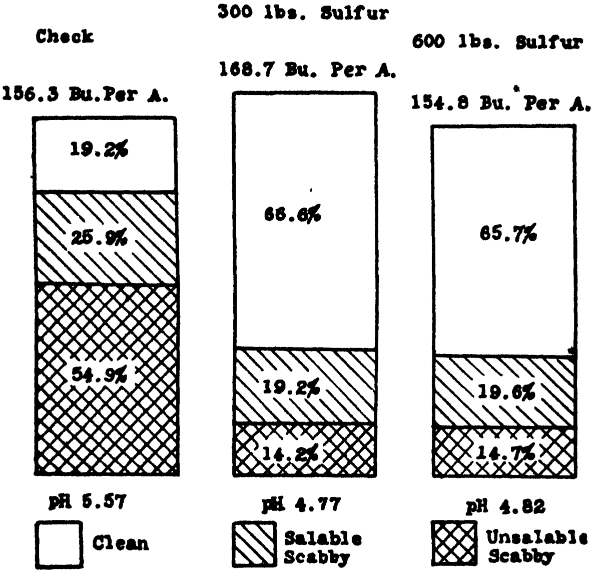

FIG. 2. DIAGRAM SHOWING THE RELATION OF SULFUR TREATMENTS TO HYDROGEN-ION CONCENTRATION AND TO THE PER CENT OF CLEAN, SALABLE AND UNSALABLE SCABBY TUBERS IN THE PRIMES—IRISH COBBLER VARIETY, EXPERIMENT II

Experiment III. The soil on which this experiment was conducted is a Penn Loam that had not been planted in potatoes for a number of years. To insure the presence of the scab organism in the soil scabby seed was planted. Sulfur applications were made at the rate of 300, 600, 900 and 1200 pounds per acre. The results of this experiment are given in table 3.

On the basis of total yield the plots treated with sulfur at the rate of 600 pounds per acre showed a decrease of 9.9 bushels per acre as compared with the check plots. Each of the other plots showed an increase in yield. This increase was 2.7 bushels, 11.9 bushels and 12.3 bushels per acre for the plots treated with 300, 900 and 1200 pounds of sulfur, respectively. In this experiment no tubers were rendered unsalable by scab. From table 3 it will be observed that the per cent of scabby tubers in the primes decreased with the increased sulfur applications, the plots receiving the highest sulfur application yielding the lowest per cent of scabby tubers.

TABLE 3

Influence of sulfur applications on total yield, per cent of scabby tubers and hydrogen-ion concentration

TREATMENT	TOTAL YIELD	YIELD OF PRIMES		YIELD OF SECONDS	pH VALUES OF SOIL EXTRACTS
		Clean	Scabby		
	bushels per acre	*bushels per acre*	*per cent*	*bushels per acre*	
Check*	144 3	128 4	60 0	14.7	6.27
300 pounds sulfur per acre†	147 0	135 4	43.6	11.6	5 90
600 pounds sulfur per acre†	134 4	119 4	26 9	15 0	5.83
900 pounds sulfur per acre†	156 2	141 8	26 3	14 1	5 13
1200 pounds sulfur per acre†	156.6	142 5	15 7	14.1	5.10

* Average of 8 plots.

† Average of 3 plots.

TABLE 4

Influence of sulfur applications on hydrogen-ion concentration of soil extracts

TREATMENT	DATE OF SAMPLING								AVERAGE
	5/19	5/29	6/14	6/24	7/18	7/24	8/2	8/16	
	pH	pH	pH	pH	pH	pH	pH	pH	pH
Check*	6 37	6 43	6 32	6.05	6 23	6.40	6.30	6 27	6 27
300 pounds sulfur per acre†	6 40	6 26	6 00	5 70	6 20	6 00	6.10	5.90	6.07
600 pounds sulfur per acre†	6 13	5 70	5 20	4 93	5 63	5 60	5 53	5 83	5 57
900 pounds sulfur per acre†	5 90	5 16	5.16	4 63	5.90	5 00	5.10	5 13	5.25
1200 pounds sulfur per acre†	5 70	5 43	4 60	4 43	5.10	4.80	4.86	5 10	5.00

* Average of 8 plots.

† Average of 3 plots.

Soil samples were taken 35 days after the sulfur applications were made and again approximately every 10 days until the crop was harvested. Water extracts of all the soil samples were made and these were tested for the hydrogen-ion concentration. The results of these tests are given in table 4. It will

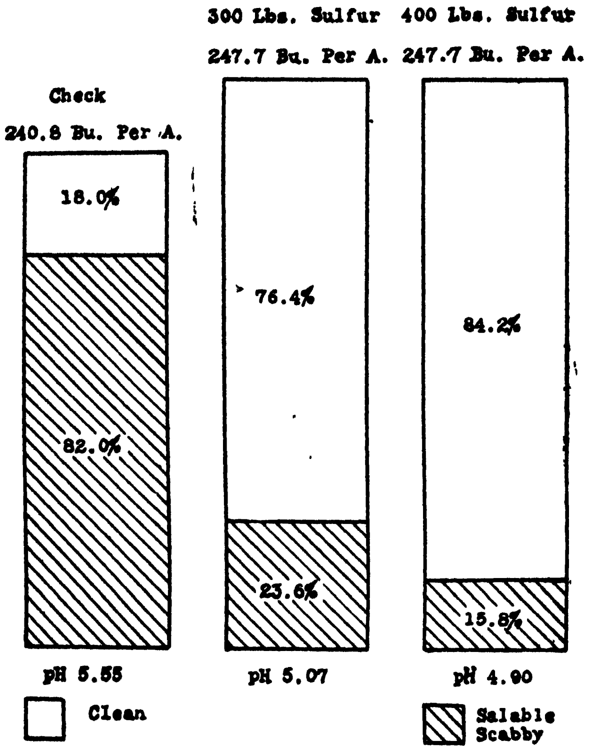

FIG. 4. DIAGRAM SHOWING THE RELATION OF SULFUR TREATMENTS TO HYDROGEN-ION CONCENTRATION AND THE PER CENT OF CLEAN AND SALABLE SCABBY TUBERS IN THE PRIMES—AMERICAN GIANT VARIETY, EXPERIMENT I

TABLE 5

Influence of sulfur applications on total yield, per cent of scabby tubers and hydrogen-ion concentration

TREATMENT	TOTAL YIELD	YIELD OF PRIMES		SECONDS	pH VALUES OF SOIL EXRACTS
		Clean	Scabby		
	bushels per acre	*bushels per acre*	*per cent*	*bushels per acre*	
Check*	280.8	240.8	82.0	40.0	5.55
300 pounds sulfur†	301 3	247.7	23.6	53.6	5.07
400 pounds sulfur†	286.6	247 7	15.8	38.9	4.90

* Average of 6 plots.

† Average of 4 plots.

hydrogen-ion exponent values of soil samples taken from the check plots was 5.55 while the corresponding exponent values for the samples from the plots treated with 300 pounds and from those treated with 400 pounds of sulfur per acre were 5.07 and 4.90, respectively. It will thus be observed from table 5 and from the diagrams of figure 4 that with each decrease in the value of the hydrogen-ion exponents below that of the check plots, a corresponding decrease occurred in the per cent of scabby tubers among the primes.

Experiment II. This experiment was conducted on a light sandy loam soil underlaid at a shallow depth by a greensand marl. In 1918 potatoes grown in the field had been severely scabbed. Sulfur was applied at the rate of 500 and 700 pounds per acre. The results are presented in table 6. The data of this table indicate a reduction of 44.8 bushels per acre for the plots treated with 500 pounds of sulfur in comparison with the corresponding yields from the check plots. It is questionable, however, whether this decrease in yield can be ascribed to the sulfur treatment, since the plots receiving the 700-pound application gave an increase of 3.5 bushels per acre over the yields from the check plots. A much lower percentage of scabby tubers was obtained from the treated than from the untreated plots. This decrease in the percentage of scabby tubers was much more pronounced than is apparent from the figures given in the table, since the tubers from the treated plots classified as scabby showed fewer lesions than did those from the check plots.

TABLE 6

Influence of sulfur applications on total yield, per cent of scabby tubers and hydrogen-ion concentration

TREATMENT	TOTAL YIELD	YIELD OF PRIMES		YIELD OF SECONDS	pH VALUES OF SOIL EXTRACTS
		Clean	Scabby		
	bushels per acre	*bushels per acre*	*per cent*	*bushels per acre*	
Check*	247.3	184.4	64.4	62.8	5.6
500 pounds sulfur†	202.5	161.2	38.8	41.3	4.8
700 pounds sulfur†	250.8	191.6	23.9	59.1	4.8

* Average of 6 plots.
† Average of 4 plots.

The relation of the hydrogen-ion exponents of the soil samples collected from the treated and untreated plots to the yields of scabby and clean tubers is shown in the diagrams of figure 5. Soil samples taken before the sulfur applications were made gave an average hydrogen-ion exponent value of 5.8. At the time of harvesting, the average hydrogen-ion exponent values of the soil samples from the check plots treated with 700 pounds and from those treated with 500 pounds of sulfur per acre was 4.8 in both cases. Despite this fact the per cent of scabby tubers among the primes harvested from the plots treated with 700 pounds was lower than from those treated with 500 pounds, being 23.8 for the former and 38.8 for the latter, as compared with 64.4 for the check plots.

In this experiment the amount of scab on the tubers from the treated plots was reduced to only a few lesions. It is doubtful whether scab could be entirely eliminated from the crop even by heavier applications of sulfur, since the exponent values resulting from the 400 and 700-pound applications are considerably lower than the exponent shown by Gillespie (7) to inhibit the growth of the scab organism in culture media.

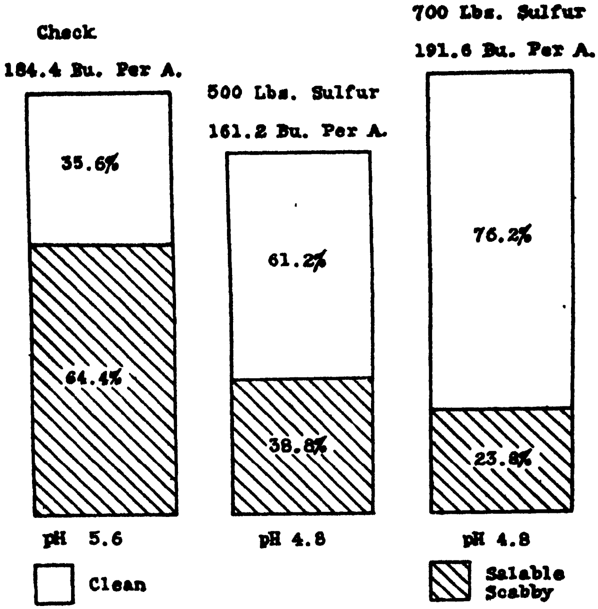

FIG. 5. DIAGRAM SHOWING THE RELATION OF SULFUR TREATMENTS TO HYDROGEN-ION CONCENTRATION AND THE PER CENT OF CLEAN AND SALABLE SCABBY TUBERS IN THE PRIMES—AMERICAN GIANT VARIETY, EXPERIMENT II

The necessity of determining the hydrogen-ion concentration of the soil before making sulfur applications is evident from the experiments here reported. In the case of three of these, soil samples taken before the sulfur applications were made showed an average hydrogen-ion concentration of 5.66. At the time of harvesting the average hydrogen-ion exponent values of the soil samples from plots receiving 300 pounds of sulfur per acre was approximately the same as where larger amounts (400 to 700 pounds) were used, being 4.92 for the former and from 4.8 to 4.9 for the latter. Scab was likewise approximately as well controlled on the plots receiving the 300-pound application as where the heavier applications were made, the percentage of scabby tubers in the primes being 28.5 for the former and from 15.8 to 38.8 for the latter. In the other two experiments soil samples taken before the sulfur applications were made showed an average exponent value of 6.26, and here the heavier applications of sulfur (400 to 1200 pounds) not only gave a greater decrease in the exponent values than the lighter applications (300 pounds) but with each decrease in the value of the hydrogen-ion exponents below that of the check plots, a corresponding decrease occurred in the per cent of scabby tubers among the primes.

SUMMARY

With due regard for the limitations of the experiments here reported, resulting from the fact that they were conducted for one year only, the following points may be advanced.

1. With the different amounts of sulfur used, all gave substantial gains in the number of clean tubers. With the heaviest application, however, scab was not entirely eliminated.
2. The results would indicate that with those varieties of potatoes known to scab severely the use of sulfur in the proper amount will render a large portion of the crop salable.
3. In all cases, following applications of sulfur there was an increase in soil acidity as measured by the hydrogen-ion concentration of soil extracts. In most instances this increase in acidity, corresponding to a decrease in hydrogen-ion exponent, was in proportion to the amount of sulfur applied.
4. With a decrease in hydrogen-ion concentration there was a decrease in the number of scabby tubers.
5. The necessity of knowing the soil reaction before sulfur applications are made is evident from the fact that where the hydrogen-ion concentration of water extracts of soil samples taken before the sulfur applications were made was 5.8 or less, the lighter applications (300 to 500 pounds) gave approximately as good control of scab as the heavier applications (700 to 1200 pounds). Where the initial exponent was greater than 6.0 the heavier applications gave the best control.
6. The results of the present work would indicate that the limiting exponent for the growth of the scab organism is lower in soil than in culture media.

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EFFECT OF VARIOUS SOLUBLE SALTS AND LIME ON EVAPORATION, CAPILLARY RISE, AND DISTRIBUTION OF WATER IN SOME AGRICULTURAL SOILS

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INTRODUCTION

Soil fertility can not be maintained if crops are removed from the land and not enough of the plant-food is returned to the soil to compensate it for the loss. In spite of some contentions to the contrary, it can not even be maintained, if a strictly live-stock type of farming is practiced, in which all crops are fed to the farm animals and manure is carefully preserved and returned to the soil. If animals themselves or animal products, such as milk, cheese, wool, eggs, etc., are removed from the farm, a considerable amount of soil fertility is carried away. As a general rule, the barnyard manure is not carefully conserved on American farms, and before it reaches the field more or less of the plant-food is lost through ammonification, leaching, etc. Moreover, there is a considerable loss of plant-food from soil in the drainage water through washing during excessive rains and leaching. If we are to preserve the present status of soil fertility, therefore, we must in some way periodically compensate soil for the constant loss in its fertility. This being established, it implies that such compensation on a national scale must be largely in the form of the application of some chemical fertilizers, the nature of which might vary considerably, depending on the character of the soil, climatic conditions, and the systems and methods of farming.

Regardless of the nature and the kind of fertilizer materials to be used most advantageously under different conditions, it is of considerable importance that the question of the effect of these materials on some soil properties should be studied.

Soil moisture is one of the most important factors in crop production. Broadly speaking, the conservation of soil moisture in order to have an ample water supply at the proper time for the germination of seed, growth and development of crops is a subject of much study and consideration among the agriculturists of to-day. The present study was undertaken with the hope of obtaining some additional information on the subject of the effect of some chemical substances on evaporation, capillary rise and distribution of water in some agricultural soils.

REVIEW OF LITERATURE ON THE EFFECT OF SOLUBLE SALTS ON EVAPORATION OF SOIL MOISTURE

There are several men who have contributed to our knowledge on the general subject of the effect of chemical compounds on the evaporation of water from soils.

Warington (18), in his book on "Physical Properties of Soils," cited Johnson and Armsby of Connecticut and King of Wisconsin, who did some work along this line. They found that "saline matter of the soil, or of additions of saline manures" very materially decreases the evaporation of soil water. The action of these materials was attributed to the fact that a slight crust was formed on the surface and completely filled up the interstices of soil. "From this point of view," adds Warington (18, p. 118), "salts of little solubility, as gypsum, should be those which most effectively hinder evaporation."

Buffum (5) in studying alkali soils of Wyoming, was impressed with the fact that all alkali soils appear damp, when adjoining lands may be almost perfectly dry. Thinking that the salts of alkali lands prevented the loss of water, he treated some normal soil in pots with solutions of various concentrations of sodium chloride, sodium carbonate, sodium sulfate, magnesium sulfate and sugar. He found that every one of these substances diminishes the evaporation, and that their effect is increased with the concentration. When about 2.2 per cent of alkali salts were present in soil, the evaporation of water from the soil decreased to about one-half, as compared with the untreated soil.

Briggs (2), working with sodium chloride, sodium carbonate and sodium sulfate, substantiated Buffum's results. Explaining the effect of soluble salts on the evaporation of soil water, he presented an excellent discussion on the rôle of surface tension, viscosity and vapor pressure as contributing to the results found. Since his quantitative results and also those of Buffum's experiments considerably exceeded those that could be justified from theoretical consideration of the three factors mentioned above, Briggs attributed them to the crystallization of salts at the surface, thus forming a mulch.

Mohr (16), in 1909, studying the rate of evaporation from water and soil surfaces, had noticed that at the beginning evaporation was greater from the soil, but later decreased until it was less than from the water surface. It would seem that this change in the rate of evaporation in case of soil might be due to the effect of soluble salts in that soil. When their amount became large enough at the surface to modify considerably the concentration of the soil solution, its effect on the rate of evaporation became pronounced.

Dumus (7) in 1911 studied the effect of some salts on evaporation of water from soil in tumblers. His results show that soluble salts decrease the evaporation.

More recently Harris and Robinson (8) have tested the effect of a solution of sodium chloride on quartz sand in small porcelain crucibles. The salt concentration was varied from half normal to 7 times normal. The evaporation was allowed to take place under a bell jar in order to avoid air currents. The results show that with the increase in concentration of sodium chloride the evaporation gradually decreased.

This brief perusal of some of the available literature on the subject shows very clearly that the soluble and insoluble salts affect to some extent the rate at which water evaporates from the soil. However, the data are not available on this subject that would tend to show how lime and some of the soluble salts which are often used in agricultural practice would influence the rate of water evaporation from some agricultural soils, when water is supplied and constantly brought from a given depth below the soil surface. Also, what relation, if any, exists between evaporation of soil water, capillary rise and eventual distribution of soil water as influenced by these chemical substances? In the present study an endeavor is made to add some data on this subject.

EFFECT OF DIFFERENT CHEMICAL SUBSTANCES ON EVAPORATION OF WATER FROM DIFFERENT SOILS

Procedure

In the present work only chemically pure substances were used for treating the soils.

After some preliminary trials it was decided to adhere to the following general procedure. Cylinders, 12 inches long and 4 inches in diameter, with the bottoms made of wire gauze, were filled uniformly and with considerable packing, with sand or an air-dried soil. Then the surface three inches of the soil was removed and thoroughly mixed with 7.6 gm. of a given salt or lime, and the soil was packed back into the cylinder. Since the surface of the exposed area in the cylinders equals 12.566 square inches, the application of 7.6 gm. of chemical substance represents approximately 1 ton to the acre. To prevent any chemical action of soil solution on the galvanized iron of the cylinders, the inner walls of the cylinders were carefully coated with melted paraffine before filling in the soil. The cylinders containing the soil, when placed in their special bases, formed units similar to evaporimeters made by the Central Scientific Company of Chicago. Distilled water was supplied from these base containers which served as reservoirs, thus keeping the base of the soil column always moist. The whole apparatus was weighed from time to time, usually every other day. Livingston's (15) porous cup atmometer was set up among the soil evaporimeters and the evaporation of water from this atmometer was observed during the period of experiments. The evaporimeters were placed in a greenhouse and the evaporation continued from 35 to 50 days.

Results and discussion

The results with sea sand, Sassafras medium sandy loam, Penn loam, Elkton clay loam, and muck, treated with ammonium sulfate, potassium sulfate, magnesium sulfate, sodium chloride, calcium nitrate, potassium diphosphate and calcium oxide, are given in tables 1, 2, 3, 4, and 5 and summarized graphically in figure 1. Later, when larger cylinders were set up for determining the effect of the application of sodium chloride, potassium monophosphate and calcium oxide on the moisture content of brown silt loam, additional data on the influence of these substances on the evaporation of water from soil were obtained. These figures, comprising an observation of a 10-day period, are given in table 6.

TABLE 1

Effect of different salts and lime on the evaporation of water from sea sand

Experiment continued 37 days

TREATMENT	CYLINDER 1	CYLINDER 2	AVERAGE	DECREASE OVER UNTREATED	WATER SAVED BY TREATMENT
	gm.	*gm.*	*gm.*	*gm.*	*per cent*
Untreated	313	355	334		
$(NH_4)_2SO_4$	113	105	109	225	65.7
K_2SO_4	132	138	135	199	59.6
$MgSO_4$	190	239	215	119	35.7
NaCl	94	156	125	209	62.6
$Ca(NO_3)_2$	93	96	95	239	71.6
KH_2PO_4	114	108	111	223	66.8
CaO	215	316	266	68	20.3
Atmometer			656		

TABLE 2

Effect of different salts and lime on evaporation of water from Sassafras medium sandy loam

Experiment continued 50 days

TREATMENT	CYLINDER 1	CYLINDER 2	AVERAGE	DECREASE OVER UNTREATED	WATER SAVED BY TREATMENT
	gm.	*gm.*	*gm.*	*gm*	*per cent*
Untreated	641	610	626		
$(NH_4)_2SO_4$	477	482	480	146	23.32
K_2SO_4	555	558	557	69	11.02
$MgSO_4$	476	495	486	140	22.37
NaCl	397	388	393	233	37.22
$Ca(NO_3)_2$	422	428	425	201	32.11
KH_2PO_4	611	548	580	46	7.35
CaO	628	579	604	22	3 51
Atmometer			708		

The results with different soils and chemicals show several interesting features. When the applications are made in the amounts used in these experiments all the above-mentioned soluble salts and the base decrease the rate of evaporation of water from the soils studied. Their effect, however, varies considerably with different soils. Generally speaking, the influence of soluble salts is most pronounced in the sea sand and least pronounced in the Penn loam. Also the depressing effect is greater in the Elkton clay loam than in the Sassafras sandy loam. Likewise, the behavior of some individual substances with different soils is worth noticing. Sodium chloride, for example, is among the most effective in checking the evaporation of water from nearly every soil studied, while potassium sulfate and potassium phosphate could be ranked as among the least effective. Ammonium sulfate and magnesium sulfate occupy rather an intermediate position in this respect. The action of lime is especially interesting. With sand, sandy loam and Penn loam, calcium oxide in the amount applied is one of the least effective; its effect on muck is relatively much more pronounced, while in clay loam it is

TABLE 3

Effect of different salts and lime on the evaporation of water from Penn loam

Experiment continued 41 days

TREATMENT	CYLINDER		AVERAGE	DECREASE OVER UNTREATED	WATER SAVED BY TREATMENT
	1	2			
	gm.	*gm.*	*gm.*	*gm.*	*per cent*
Untreated	966	953	960		
$(NH_4)_2SO_4$	734	751	743	217	22.6
K_2SO_4	867	832	850	110	11.5
$MgSO_4$	789	780	785	175	18.2
$NaCl$	761	741	751	209	21.8
$Ca(NO_3)_2$	802	779	791	169	17.6
KH_2PO_4	902	888	895	65	6.8
CaO	841	875	858	102	10.6
Atmometer			697		

TABLE 4

Effect of different salts and lime on the evaporation of water from Elkton clay loam

Experiment continued 35 days

TREATMENT	CYLINDER		AVERAGE	DECREASE OVER UNTREATED	WATER SAVED BY TREATMENT
	1	2			
	gm.	*gm.*	*gm.*	*gm.*	*per cent*
Untreated	917	882	900		
$(NH_4)_2SO_4$	542	638	590	310	34.4
K_2SO_4	590	698	644	256	28.4
$MgSO_4$	570	576	573	327	36.3
$NaCl$	383	385	384	516	57.3
$Ca(NO_3)_2$	441	467	454	446	49.6
KH_2PO_4	485	629	557	343	38.1
CaO	232	343	288	612	68.0
Atmometer			590		

the most efficient in decreasing the evaporation of water, as compared with the salts studied.

The relative rate of evaporation of moisture in the lime and salt treated soils can be studied in figure 1 and also figure 4. In the latter figure, in which ammonium sulfate, sodium chloride, and calcium oxide were compared in sandy loam and clay loam, the relative position of soils treated with different substances is worthy of consideration. In comparison with the untreated soil, ammonium sulfate and sodium chloride occupy the same general position in relation to one another. The curve of the limed sandy soil, however, runs next to the curve of the untreated soil, while the curve of the limed clay loam drops to the lowest place. The respective data for soils treated with potassium phosphate (see tables 15 and 16) also show a pronounced difference. In clay loam the effect of this salt is equal to that of ammonium sulfate, while in sandy loam its effect is considerably smaller than that of the latter salt.

TABLE 5

Effect of different salts and lime on the evaporation of water from muck

Experiment continued 41 days

TREATMENT	CYLINDER 1	CYLINDER 2	AVERAGE	DECREASE OVER UNTREATED	WATER SAVED BY TREATMENT
	gm.	*gm.*	*gm.*	*gm.*	*per cent*
Untreated	1014	900	957		
$(NH_4)_2SO_4$	765	767	766	191	20.0
K_2SO_4	888	921	905	52	5 4
$MgSO_4$	896	801	849	108	11.3
NaCl	437	318	378	579	60.5
$Ca(NO_3)_2$	486	562	524	433	45.5
KH_2PO_4	672	698	685	272	28.8
CaO	612	533	573	384	40.3
Atmometer			567		

TABLE 6

Effect of different chemical substances on evaporation of water from brown silt loam; data represent grams of water evaporated from cylinders in 10 days and the amount and per cent of water saved by each treatment

TREATMENT	CYLINDER 1	CYLINDER 2	AVERAGE	DECREASE DUE TO TREATMENT	
	gm	*gm.*	*gm.*	*gm.*	*per cent*
Untreated	577	618	598		
NaCl	390	398	394	204	34.11
K_2HPO_4	558	554	556	42	7.02
CaO	507	551	529	69	11.54

In connection with the results obtained the inquiry naturally arises as to why different chemical substances act so differently, when applied to the same soil, and why the behavior of lime, as well as that of potassium phosphate, is apparently different in different soils.

In the attempt to answer the general inquiry several factors must be considered as the agencies contributing to the variations in the effect of different chemical substances on various soils studied.

With an increase in the concentration of the salt solution the vapor pressure is decreased. This well known fact of physical chemistry is of prime importance in understanding the effect of soluble salts on the evaporation of water from soils, for the vapor pressure is defined by a physical chemist as the "tendency shown by the substances to pass from the liquid or solid into the gaseous state," i.e., for evaporation of water from solution or soil surface. It is logical to suppose that this general law is applicable to soil solutions with some possible modifications brought about by any physical or chemical changes in soil due to the introduction of these chemical substances to the soil.

Again, different salts of the same absolute concentration vary considerably in their property to decrease the vapor pressure of the resultant solution. Some of the salts studied in the present work have, according to Tammann (17), the following order of their efficiency in this respect: $NaCl > (NH_4)_2SO_4 > K_2SO_4 > KH_2PO_4 > MgSO_4$. For the lack of more direct data bearing on the subject studied, this general fact might be helpful in understanding the results obtained on the evaporation of water from soils treated with these salts.

When a given soluble salt, or a solution of this salt, is added to the soil, the concentration of soil solution is increased. The extent of the increase in concentration of soil solution varies very greatly, depending on the salt or the texture of the soil. Bouyoucos and McCool (1), using $0.1N$ solutions of different salts with different soils have shown that efficiency in increasing the concentration of the soil solution was greater in light sandy soils and decreased with the heavier types of soil. For the individual salts the efficiency followed generally the following order, with the salts studied in the present work: $Ca(NO_3)_2 > (NH_4)_2SO_4 > MgSO_4 > K_2SO_4 > K_2HPO_4$. The difference between magnesium sulfate and potassium sulfate is not so striking, while the relative gradation from calcium nitrate to potassium phosphate is very pronounced. Referring again to tables 1, 2, 3, 4 and 5, or to the graphical representation in figure 1, one observes that the order of efficiency in depressing the water evaporation in soils for the salts mentioned above is very similar to the order of their efficiency in increasing the concentration of the soil solution, if added in solutions of $0.1N$ concentration. In Sassafras sandy loam and muck this order is calcium nitrate > ammonium sulfate > magnesium sulfate > potassium sulfate > potassium phosphate. In Elkton clay loam ammonium sulfate changes places with magnesium sulfate; the remaining salts retain their position. In sea sand magnesium sulfate is less effective than potassium sulfate, the positions of the remaining salts being unchanged.

DISTRIBUTION OF SOLUBLE SALTS IN TREATED SOILS

The relation of the effect of different salts on water evaporation in our experiments could not be expected to be entirely parallel with the effect of these salts on the concentration of soil solution, when applied in concentrations equal to $0.1N$, because the rate of application in our experiment was widely different from that in Bouyoucos and McCool's experiment. Yet, the parallel is very striking.

In order to verify this important fact, the concentration of the soil solution at different depths of the soil cylinders was determined with the use of the freezing-point method (1). Determinations were made at the end of the experiment, adhering to the following general procedure.

Procedure

The soil from one of the two cylinders of each treatment was taken out in 1-inch layers down to 6 inches, air-dried, and to a given sample of soil enough water was added to make it into a conveniently workable mud ball. For a given soil the ratio of soil to water remained constant. For the sea sand 25 gm. of sand and 4 cc. of distilled water were used; for Elkton clay loam 20 gm. of soil and 6 cc. of water; for Penn loam, 20 gm. of soil and 6.5 cc. of water; and 10 gm. of muck was mixed with 10 cc. of water. These quantities of prepared material were placed in glass tubes, and freezing-point determinations were made. The soil in the second of the two cylinders of each treatment was flushed with 2 inches of distilled water (412 cc. per 12.566 square inches of the exposed surface of the soil) and allowed to drain; likewise, 1-inch layers of soil were taken out, dried and the concentrations of the soil solution determined by the same method. After every two or three duplicate samples of soil the freezing-point determination of distilled water was made.

Results and discussion

The results are recorded in tables 7, 8, 9 and 10, an examination of which reveals a very interesting relation of the effect of different substances on the evaporation of water from soils. Remembering that all these substances were applied to the upper 3 inches of the soil, the data show that at the end of the experiment most of the salts were brought to the surface and there deposited. It may be mentioned in passing that the results show that practically no diffusion of salts took place downward against the rise of capillary water. Sodium chloride affords an exception in both sand (in the fourth inch) and muck (fourth and fifth inches), where slight diffusion evidently took place during the 37 days of the duration of the experiment.

If we will consider the osmotic concentrations of the soil solution in the surface inch of soil of the unleached cylinder for different substances, as illustrated in figure 2, and compare the order of the magnitudes of these concentrations with the depressive effect of different substances on the evaporation of water from the same soil, we find that these values run in parallel fairly well.

TABLE 7

Concentration of soil solution of sea sand at different depths in the evaporimeters at the end of experiment

The determinations were made both before and after leaching the sand with 2 inches of water; data represent average of two determinations; 25 gm. of sand and 4 cc. of distilled water mixed together for the test

TREATMENT	DEPTH FROM SURFACE											
	1 inch		2 inches		3 inches		4 inches		5 inches		6 inches	
	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure
	°C	atm	°C	atm	°C	atm	°C.	atm	°C.	atm.	°C.	atm.
Untreated												
Unleached	0 030	0 36	0 006	0 07	0 007	0 08	0 011	0 13	0 010	0 12	0 007	0.08
Leached ..	0 006	0 07	0 008	0 10	0 008	0 10	0 011	0 13	0 011	0 13	0 011	0.13
$(NH_4)_2SO_4$												
Unleached .	2 030	24 40	0 071	0 86	0 061	0 74	0 034	0 41	0 019	0 23	0 016	0.19
Leached	0 349	4 21	0 136	1 53	0 103	1 24	0 083	1 00	0 080	0 97	0 076	0.92
K_2SO_4												
Unleached .	1 830	22 00	0 075	0 90	0 030	0 36	0 017	0 20	0 015	0 18	0.015	0 18
Leached	0 160	1 93	0 045	0 54	0 046	0 55	0 044	0 53	0 046	0 55	0 045*	0 54
$MgSO_4$												
Unleached.	1 226	14 75	0 012	0 15	0 011	0 13	0 066	0 07	0 004	0 05	0 003	0.04
Leached . .	0 410	0 49	0 041	0 49	0 042	0 51	0 040	0 48	0 040	0 48	0 038	0 46
NaCl												
Unleached	2.582	31 00	0 398	4 80	0 088	1 06	0 031	0 37	0 004	0 05	0 005	0.06
Leached . ..	1 684	20 25	0 167	2.01	0 141	1 70	0 158	1 91	0 177	2.13	0 173	2 09
$Ca(NO_3)_2$												
Unleached	2 808	33 70	0 301	3 63	0.021	0 25	0 017	0 20	0 014	0 17	0 010	0 12
Leached.. .	0 194	2 34	0 098	1.15	0 096	1 16	0 088	1 06	0 063	0.76	0 045	0 54
K_2HPO_4												
Unleached	1 797	21 30	0.058	0.70	0.022	0 27	0 009	0 11	0 009	0 11	0.011	0.13
Leached....	0.168	2 03	0 089	1 07	0.068	0 82	0.055	0 66	0 052	0 63	0 056	0 68
CaO												
Unleached....	0 082	0 99	0 021	0 25	0 010	0 12	0.011	0 13	0 006	0.07	0 009	0 11
Leached......	0.050	0.60	0 041	0 49	0 036	0 43	0 037	0 45	0 031	0 37	0 022	0.27

* One determination.

For the sake of simplicity let us temporarily exclude from discussion potassium phosphate and lime. Then in sea sand the order of magnitude of osmotic concentrations for the different treatments is calcium nitrate > sodium chloride > ammonium sulfate > potassium sulfate > magnesium sulfate, and this is exactly the order of depressive effect of these salts on evaporation (fig. 1). For Elkton clay loam and muck the order for osmotic concentrations is sodium chloride > calcium nitrate > ammonium sulfate > potassium sulfate > magnesium sulfate. The order of their effectiveness in retarding the evaporation is also the same with the exception of magnesium sulfate. The latter salt in clay and muck soils in this respect is more effective than potassium sulfate. This fact, moreover, is brought out by every soil studied (fig. 1), sand being an

TABLE 8

Concentration of Elkton clay loam at different depths in the evaporimeters at the end of experiment

The determinations were made both before and after leaching the soil with 2 inches of water; data represent average of two determinations; 20 gm. soil and 6 cc. of distilled water mixed together for the test

TREATMENT	DEPTH FROM SURFACE											
	1 inch		2 inches		3 inches		4 inches		5 inches		6 inches	
	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure
	°C.	atm	°C.	atm.	°C.	atm	°C.	atm.	°C.	atm.	°C.	atm.
Untreated												
Unleached ...	0 156	1.88	0 020	0.24	0 018	0 21	0 019	0 23	0 018	0.22	0 018	0.22
Leached	0 114	1.38	0 032	0 39	0 028	0 34	0 020	0 24	0 021	0 25	0.020	0.24
$(NH_4)_2SO_4$												
Unleached	1 323	15.92	0 086	1.04	0 051	0 62	0 046	0 55	0 014	0.17	0 013	0.16
Leached	0 634	7.64	0 239	2 88	0.164	1.98	0 134	1 51	0 101	1 22	0 037	0 45
K_2SO_4												
Unleached	0 897	10 80	0 052	0 63	0 025	0.30	0 022	0 27	0 017	0.20	0 017	0 20
Leached	0 509	6.13	0 094	1 13	0 097	1.17	0 056	0 68	0 058	0 70	0 057	0 69
$MgSO_4$												
Unleached ...	0 449	5 41	0.049	0 59	0 038	0 46	0 020	0 24	0 019	0.23	0.018	0.22
Leached	0 102	1.23	0 086	1 04	0 136	1.53	0 133	1.49	0 080	0 97	0 073	0.88
NaCl												
Unleached	4 043	48 60	0 161	1 94	0 067	0 81	0 026	0.31	0 022	0 27	0 016	0.19
Leached ...	2.152	24.76	0 599	7 22	0 137	3 82	0 263	3.17	0 260	3 13	0.235	2.83
$Ca(NO_3)_2$												
Unleached. ..	2.094	25 16	0 055	0 66	0 029	0 35	0 021	0.25	0 021	0.25	0.021	0.25
Leached	1.200	14.44	0 284	3 42	0 232	2.80	0.172	2.07	0 142	1.71	0.119	1.44
K_2HPO_4												
Unleached	0 238	2 87	0 043	0 52	0 020	0 24	0 011	0.13	0 009	0.11	0 010	0 12
Leached	0.162	1.95	0 062	0 75	0 035	0 42	0.029	0.35	0 029	0.35	0.025	0 30
CaO												
Unleached ...	0 114	1.38	0 034	0 41	0 033	0 40	0 014	0.17	0 014	0.17	0.013	0.16
Leached	0 066	0 80	0.053	0 64	0 051	0 62	0 045	0.54	0.046	0 55	0.038	0 46

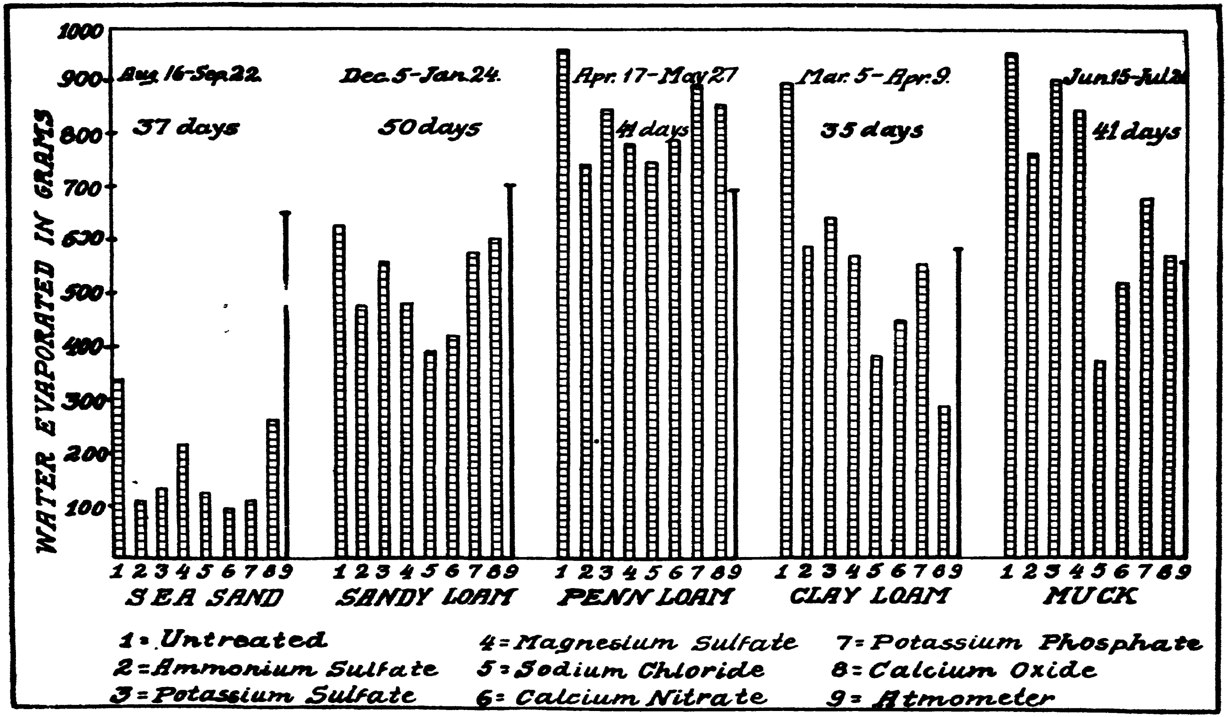

FIG 1. INFLUENCE OF VARIOUS SALTS AND LIME ON THE EVAPORATION OF WATER FROM DIFFERENT SOILS AND SAND

The dates show the time of the year when the experiment was conducted and its duration. With the exception of no. 9, which represents the atmometer, the numbers correspond to those on the soil cylinders with the various treatments exception. Perhaps the behavior of this salt is due to its effect on the physical properties of soils studied. In sand the salts probably were practically unchanged (with the exception of ammonium sulfate, which perhaps was partly nitrified). In the soils, however, some reactions took place, such as double decomposition, exchange of bases, absorption and adsorption. Some of these reactions, undoubtedly, have very considerably modified the soil solution qualitatively; they also affected the physical conditions of the soils. This latter effect is also somewhat noticeable in the case of potassium phosphate and especially in that of calcium oxide. Only in sea sand the applied potassium phosphate retained a considerable concentration of the soil solution. In both Elkton clay loam and muck the soil solution with this salt showed a very slight increase in concentration over the untreated soil. The decrease in evaporation of water due to this salt was unproportionally large, as one would notice by comparing the data in figures 1 and 2 for potassium sulfate and potassium phosphate.

TABLE 9

Concentration of soil solution of Penn loam at different depths in evaporimeters at the end of experiment

The determinations were made both before and after leaching the soil with 2 inches of water; data represent average of two determinations; 20 gm. of soil and 6½ cc. of distilled water mixed together for the test

TREATMENT	DEPTH FROM SURFACE											
	1 inch		2 inches		3 inches		4 inches		5 inches		6 inches	
	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure
Untreated	*°C.*	*atm.*	*°C.*	*atm*	*°C*	*atm*	*°C.*	*atm.*	*°C.*	*atm*	*°C.*	*atm.*
Unleached...	0 151	1.82	0 018	0.22	0 014	0 17	0 009	0 11	0.008	0 10	0 008	0.10
Leached.. .	0 130	1 46	0 027	0 33	0 016	0 19	0 005	0 06	0 006	0 07	0 008	0.10
$(NH_4)_2SO_4$												
Unleached...	1 691	20 33	0.143	1 72	0 043	0 52	0 009	0 11	0 011	0.13	0 010	0.12
Leached ...	0 615	7.41	0 232	2 80	0 116	1.40			0 064	0 77		
CaO												
Unleached .	0 099	1 19	0 023	0 28	0 019	0 23	0 019	0.23	0 018	0 22	0.016	0.19
Leached.....	0 057	0 69	0 033	0 40	0 019	0 23			0.016	0 19	0.016	0.19

If we are going to apply the same criterion in the study of the behavior of calcium oxide, the results obtained are still more striking. The concentration of the soil solution of soils or sand treated with this substance is very small, in all three soils studied it was even slightly lower than the concentration of the soil solution in the untreated cylinder. Its effect on evaporation is slight in sandy loam, larger in Penn loam, still greater in muck, and was very great indeed in Elkton clay loam. In this latter soil its influence was greater than that of any other salt tried. There is a very strong possibility that the effect of calcium oxide on the change in physical condition of the clay soil was responsible for such enormous decrease in the evaporated water.

TABLE 10

Concentration of soil solution of muck at different depths in the evaporation at the end of experiment

The determinations were made both before and after leaching the sand with 2 inches of water; data represent average of two determinations; 10 gm. of muck and 10 cc. of distilled water used for the test

TREATMENT	DEPTH FROM SURFACE											
	1 inch		2 inches		3 inches		4 inch		5 inches		6 inches	
	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure	Freezing-point depression	Osmotic pressure
Untreated	°C.	atm	°C	atm	°C	atm	°C.	atm	°C	atm	°C	atm
Unleached	0 230	2 77	0 061	0 74	0 060	0 72	0 063	0 76	0 062	0 75	0 062	0.75
Leached	0 140	1 69	0 141	1 70	0 088	1 06	0 045	0 54	0 035	0 42	0 034	0 41
$(NH_4)_2SO_4$												
Unleached	0 824	8 92	0 255	3 07	0 121	1 46	0 080	0 97	0 064	0 77	0.060	0 72
Leached	0 367	4 42	0 448	5 40	0 297	3 58	0 110	1 33	0 046	0 55	0 038	0 46
K_2SO_4												
Unleached	0 568	6 84	0 109	1 32	0 074	0 89	0 074	0 89	0 068	0.82	0.067	0.81
Leached	0 236	2 84	0 290	3 50	0.219	2 64	0 160	1.93	0 094	1.13	0 055	0 66
$MgSO_4$												
Unleached	0 269	3 24	0 083	1 00	0 082	0 99	0 076	0 92	0 065	0 78	0 057	0 69
Leached	0 131	1 47	0 133	1 49	0 132	1 48	0 093	1 12	0 057	0 69	0.040	0 48
NaCl												
Unleached	2 242	26 93	0 957	11 52	0 406	4 89	0 218	2 63	0 106	1 28	0.054	0 65
Leached	0 225	2 71	0 592	7.13	1 064	12 81	0 935	11 26	0 708	8 53	0 435	5.24
$Ca(NO_3)_2$												
Unleached	1 358	16 34	0 124	1 50	0 075	0 90	0 068	0 82	0 068	0 82	0.066	0.80
Leached	0 388	4 68	0 680	8 19	0 455	5 48	0 150	1.81	0 054	0 65	0 054	0 65
K_2HPO_4												
Unleached	0 286	3 45	0 [illegible]	1 11	0 069	0 83	0 070	0 84	0 066	0 80	0 068	0 82
Leached	0 101	1 22	0 144	1 73	0.113	1 36	0 096	1 16	0 061	0 74	0 050	0 60
CaO												
Unleached	0 180	2 17	0.056	0 68	0 054	0 65	0 062	0 75	0 058	0 70	0 055	0.66
Leached.	0 080	0 97	0 090	1 09	0.091	1 10	0 062	0 75	0 062	0 75	0 040	0.48

Further, in connection with the results on the determination of solution concentrations in the different soils (tables 7, 8, 9 and 10) it might be mentioned that the salts are fairly well washed down with the 2 inches of water. Even in Elkton clay loam these salts in all cases go down farther than 6 inches.

EFFECT OF CHEMICAL SUBSTANCES ON THE CAPILLARY RISE OF WATER IN SOILS

Besides the physico-chemical phenomena of the resultant soil solution being responsible for the observed behavior of these substances in checking the evaporation of water from soils, there should be mentioned another factor that might influence the evaporation of water. As it was pointed out previously, the substances were mixed in the 3-inch layer of the soil. Thus the water had to pass through the soil with these different substances before reaching the surface. It is possible, therefore, that their influence on the capillary rise of water was partly responsible for their final effect on the evaporation.

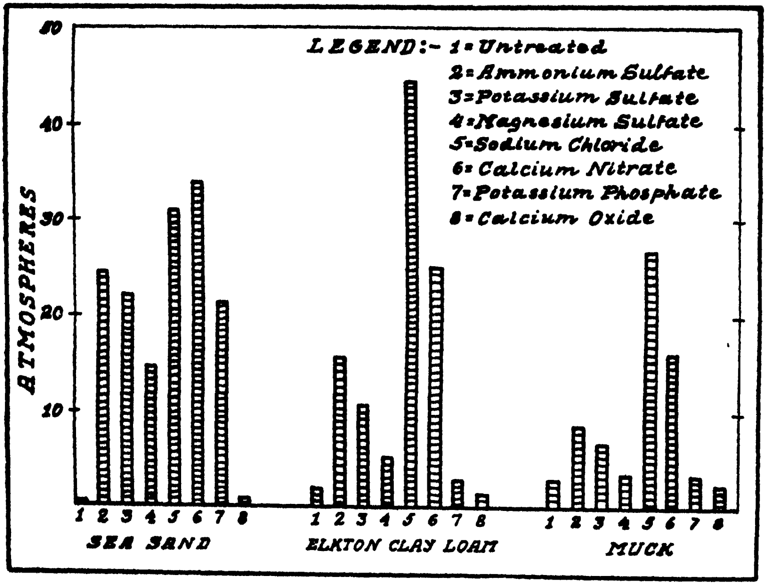

FIG. 2. OSMOTIC CONCENTRATION OF THE SOIL SOLUTION OF THE SURFACE INCH OF DIFFERENT SOILS IN CYLINDERS UNDER VARIOUS SALT TREATMENTS

Review of literature

The effect of chemical substances on the capillary rise of water in soil has been studied by several investigators. Wollny (20) found that salts which are most readily adsorbed by soils produce little effect on the capillary movement of water, while non-adsorbed salts cause a depression in capillarity, the effect being increased with the concentration.

Kravkov (14), using solutions of K_2HPO_4, Na_2HPO_4, K_2SO_4, $(NH_4)_2SO_4$, $NaNO_3$, NaCl and Na_2CO_3 of 1N, 0.2N and 0.1N concentrations with a sandy soil in glass tubes 45 cm. in height, found that all salts decreased the capillary rise of water, as compared with the distilled water. The depressive action of each salt increased with the concentration of the solution. The order in which water reached the top of the tubes for various soluble salts was as follows: $H_2O > NaNO_3 > NaCl > Na_2CO_3 > K_2SO_4 > (NH_4)_2 SO_4 > K_2HPO_4 > Na_2HPO_4$. For two insoluble salts the order was as follows: $CaSO_4 > CaCO_3 > H_2O$. Thus it is shown that phosphates, being the ones that would be absorbed by soil most readily, caused the greatest retardation, while chloride and nitrate of sodium (the least absorbed) had the least influence. The insoluble salts, such as gypsum and calcium carbonate, hastened the capillary rise, as compared with the distilled water. These results are contradictory to the findings of Wollny.

King (11), working with an 0.08 per cent solution of potassium nitrate, found that tubes filled with moist soil when treated with the salt solution lost 22.8 per cent more water than the untreated soil. Since the water was supplied from below in 18-inch tubes, the data show that the application of salt caused both increased capillary rise and evaporation. As a result of later studies by both King himself and others, he takes the opposite view and gives (12) a theoretical reasoning for the retarding action of soluble salts upon capillary movement and evaporation of soil water.

Briggs and Lapham (3) studied the effect of three sodium salts, NaCl, Na_2CO_3 and Na_2SO_4, on the capillary rise of water in Sea Island (fine sandy loam) soil, using in parallel 0.5*N* and saturated solutions of these salts. The results show that in the case of 0.5*N* solutions sodium chloride and sodium sulfate caused very little depression in the capillary rise, while sodium carbonate slightly accelerated it. When the saturated solutions were used the first two salts caused very considerable retardation in capillary movement, while sodium carbonate was effective to a lesser degree, though causing a depression. These authors explained the action of sodium chloride and that of sodium sulfate by "the resistance to a tangential shearing stress presented by the thin layer of liquid near its bounding edge" (3, p. 7) when that liquid contains a considerable amount of salt. The action of sodium carbonate was explained by the fact that this salt, being a product of strong base and weak acid, slightly hydrolyzes in water solution. Sodium hydroxide. thus formed, acts on the oily substances of soil producing the water-soluble sodium salt or soap. This cleans the surface of the soil particles, causing its easier and quicker wetting by the rising water. In conclusion the authors make an interesting deduction that "all salts which undergo an alkaline hydrolysis, viz., potassium and sodium carbonates, borates, phosphates, etc.," should affect the capillary power of soils in a similar way, as noticed in the case of sodium carbonate.

Kossovich (13) in 1910, studying sodium chloride and sodium carbonate in decinormal solutions on sand, loess clay and moor clay, found that in this concentration the influence of salts was not marked on the sand but more pronounced on the clays. In general, sodium chloride was found to hasten the rise of water, while sodium carbonate hindered the process.

Investigating the same general question, Davis (6) used K_2CO_3, KCl, P_2O_5, $KHSO_4$, $CaSO_4$, $CaHPO_4$, NH_4NO_3, and mixtures of P_2O_5 with K_2CO_3, P_2O_5 with $KHSO_4$ and $CaSO_4$ with $CaHPO_4$ on silt loam soil. He concluded that potassium bisulfate and a mixture of phosphoric acid with potassium bisulfate lower the rate of capillary flow, that the influence of phosphoric acid and potassium chloride is very insignificant, and that the other salts and mixtures studied with this soil accelerate the movement of capillary water.

In view of the conflicting results, as observed by different investigators mentioned above, it was deemed advisable to test several of the substances used in the evaporation experiments as to their action on some agricultural soils. It was decided to use NaCl as one of the least absorbed salts, K_2HPO_4 for the most absorbed salt, $(NH_4)_2SO_4$ for the intermediate, and CaO for the least soluble substance used. Soils used were Brown silt loam, typical prairie soil, and Drab clay, as the heavy type of soil. With $(NH_4)_2SO_4$ Elkton clay loam, the same soil as was used in the evaporation experiments, was employed.

Procedure

With brown silt loam and drab clay the procedure of the experiment was as follows: Either lime or the different salts were mixed with the air-dried soil at the rate of 1 per cent, which corresponded with the proportions in the evaporimeters, where applications at the rate of 1 ton per acre were made to the first 3 inches of soil. The glass tubes, 24 inches high and 1.75 inches

TABLE 11

Effect of different chemical substances on the capillary ris· of water in brown silt loam

Data represent height of rise of water in inches

DAYS	UNTREATED			NaCl			$(NH_4)_2SO_4$			K_2HPO_4			CaO		
	1	2	Average	1	2	Average	1	2	Average	1	2	Average	1	2	Average
	in	*in*	*in*	*in*	*in*	*in*	*in.*	*in*	*in.*	*in*	*in.*	*in.*	*in*	*in*	*in*
1	10 1	10 5	5 3	5 5	5 5	5 5	10 4	10 6		11.7	11 9	11.8	11 4	11 3	11 4
2	14 5	15 2	14 9	7 1	7 2	7 2	14 7	14 9	14 8	17 0	17 2	17 1	15.6	15.4	15 5
3	17.0	17 7	17 4	8 1	8 1	8 1	17 6	17 6	17 6	20 1	20 4	20 3	18 2	17 9	18 1
4	18 6	19 3	19 0	8 4	8 5	8 5	19 2	19 2	19 2	22 0	22 2	22 1	19 5	19 3	19 4
5	19 8	20 5	20 2	9 9	9 0	9 0	20 4	20 5	20 5	23 3	23 3	23 3	20 6	20 4	20 5
6	20 9	21 4	21 2	9 5	9 7	9 6	21 7	21 6	21 7				21.6	21.5	21.6
7	21 3	22 1	21.7	10 2	10 4	10 3	22 6	22 5	22 6				22.2	22.1	22.2
10				10 9	11 2	11 1									
15				12 2	12 5	12.4									
20				13.8	14.1	14.0									
25				14.8	15.1	15.0									
30				15.7	16.0	15.9									
35				16.5	16.8	16.7									
45				18.0	18.1	18 1									
55				19 4	19.5	19 5									

in diameter, were filled and uniformly packed with the treated and untreated soil. A double thickness of cheese-cloth was tied around the lower end of each tube. The soil tubes were set in a trough, in which there was a constant circulation of tap water. The height to which water rose in the tubes of different treatments, as observed periodically, is reported in tables 11 and 12. In the test with Elkton clay loam 2 per cent of ammonium sulfate was used. The procedure in this case was practically the same with the exception that the inside diameter of the tubes was 1 inch and that distilled water was employed instead of tap water. The results are recorded in table 13.

TABLE 12

Effect of different chemical substances on the capillary rise of water in drab clay

Data represent height of rise of water in inches

DAYS	UNTREATED			NaCl			$(NH_4)_2SO_4$			K_2HPO_4			CaO		
	1	2	Average	1	2	Average	1	2	Average	1	2	Average	1	2	Average
	in	*in.*	*in.*	*in*	*in*	*in*	*in*	*in*	*in*	*in*	*in*	*in.*	*in.*	*in.*	*in.*
1	2 3	2 1	2 2	0 8	0 8	0 8	2 0	2 1	2 1	2 5	3 1	2 8	6 0	5 2	5 6
2	3 2	3 1	3 2	1 3	1 2	2 3	3 1	3 2	3 2	3 3	4 1	3 7	7 6	6 7	7.2
3	3 9	3 7	3 8	1 6	1 6	1 6	3 6	3 8	3 7	4 0	4 7	4 4	8 3	7.5	7.9
4	4 3	4 1	4 2	2 0	1 9	2 0	4 1	4 3	4 2	4 3	5 1	4 7	8 7	7 9	8 3
6	5 0	4 8	4 9	2 4	2 5	2 5	4 8	5 0	4 9	4 9	5 7	5 3	9 3	8 6	9 0
8	5 4	5 1	5 3	2 8	2 8	2 8	5 4	5 5	5 5	5 3	6 0	5 7	9 6	8 9	9.3
10	5 8	5 5	5 7	3 2	3 1	3 2	5 8	5 9	5 9	5 5	6 2	5 9	9 8	9.1	9 5
12	6 3	5 9	6 1	3 5	3 5	3 5	6 2	6 4	6 3	6 0	6 8	6 4	9 8	9 3	9 6
14	6 6	6 2	6 4	3 7	3 7	3 7	6 5	6 8	6 7	6 3	7 [illegible]	6 7	10 0	9 4	9.7
16	7 0	6 5	6 8	4 0	3 9	4 0	6 9	7 1	7 0	6 6	7 3	7 0	10 0	9 4	9.7
18	7 3	6 8	7 1	4 3	4 3	4 3	7 2	7 3	7 3	7 0	7.7	7 4	10 1	9 5	9.8
21	7 7	7 2	7 5	4 7	4'6	4 7	7 7	7 8	7 8	7 3	8 0	7 7	10 2	9 6	9.9

Results and discussion

It is interesting to note that in the brown silt loam (table 11) sodium chloride caused a very marked depression in the capillary rise of water, and potassium phosphate slightly accelerated it, while neither ammonium sulfate nor calcium oxide had any pronounced influence. The action of sodium chloride and ammonium sulfate in drab clay (table 12) was very similar to their action on brown silt loam, namely, the first one retarded the capillary movement of water, while the second one had no influence on it. Potassium phosphate, unlike in its action on brown silt loam, had no effect on the rate of capillary flow of water. Calcium oxide, on the other hand, considerably accelerated the rise of capillary water, the capillary action being mostly in the first two or three days, almost stopping toward the end of the experiment.

In the light of the present knowledge of absorption of salts by soils the results with sodium chloride were not unexpected. This salt, being but little absorbed, would greatly increase the viscosity of the soil solution, thus causing a retardation in the movement of capillary water. This general rule, however, evidently does not hold true in the case of ammonium sulfate, which, according to the determination of the osmotic pressure of the soil solution of the treated soil (tables 7, 8, 9 and 10), greatly increased the concentration of soil solution in every soil studied. We may very conveniently apply Briggs and Lapham's (3) explanation for the action of potassium phosphate, which is based on the assumption that potassium phosphate hydrolizes in the soil, forming potassium hydroxide. The latter base acts on the oily substances in the soil, dissolves them, forms soap, and thus assists in the wetting of the soil by the soil solution. The same explanation, however, can not be applied to the action of ammonium sulfate.

TABLE 13

Effect of ammonium sulfate (2 per cent) on the capillary rise of water in Elkton clay loam

Data represent height of water rise in inches

DAYS	$(NH_4)_2SO_4$			UNTREATED		
	1	2	Average	1	2	Average
	in.	in.	in.	in.	in.	in.
1	8.5	7.5	8.0	10.0	10.8	10.4
2	10.5	9.0	9.8	12.8	13.3	13.1
3	11.5	10.3	10.4	14.0	14.8	14.4
4	12.7	11.0	11.9	15.8	16.3	16.1
6	14.3	12.5	13.4	18.0	18.3	18.2
8	15.3	13.8	14.6	19.5	19.8	19.7
10	16.3	14.8	15.6	20.8	21.3	21.1
12	17.5	15.8	16.7	22.8	22.8	22.8
14	18.0	16.3	17.7	24.0	24.0	24.0
19	19.0	17.5	18.3	25.5	25.5	25.5
26	20.5	19.0	19.8	28.3	28.3	28.3
30	21.3	19.8	20.6	29.3	29.3	29.3

The action of calcium oxide on the physical properties of drab clay was evidently responsible for the acceleration of water movement. The behavior of the water curve in the lime tubes was very similar to that which could be expected in sandy soil. It is possible that the coagulating action of lime on the colloids of the drab clay produced a granular structure which resulted in a reduction of the internal surface similar to that found in sandy types of soil. Being a lighter type, brown silt loam was not sufficiently modified by lime in this respect to cause the change in its property of capillarity.

Referring now to table 13, one notices that in this case the application of 2 per cent ammonium sulfate to Elkton clay loam caused a very pronounced decrease in the capillary rise of water, as compared with the untreated soil.

CORRELATION BETWEEN ACTION OF DIFFERENT SALTS IN EVAPORATION AND CAPILLARY RISE OF WATER

In studying tables 1, 2, 3, 4, 5, 11, 12 and 13, one notices but very little correlation between evaporation from soils under different treatments and the capillary rise of water in some soils. It is true that sodium chloride, one of the most efficient in depressing the evaporation of water, causes a considerable decrease in the capillary rise of water. This case, however, is rather an exception and not a rule. Neither ammonium sulfate, potassium phosphate, nor calcium oxide show any such correlation between these two phenomena. Ammonium sulfate caused considerable decrease in the evaporation of water, but was without influence on the capillary rise, when applied at the rate of 1 per cent, and displayed its action only when applied at the rate of 2 per cent. The lack of correlation in the case of potassium phosphate and calcium oxide is still more pronounced. The first substance in the case of brown silt loam and the second one in the case of drab clay caused an acceleration of capillary rise of water, yet both these substances caused a decrease in the evaporation of water in the respective types of soil. In this respect the action of lime is most interesting. It caused the greatest decrease in the amount of evaporated water in clay soil in spite of the fact that in the similar soil it increased the capillary rise of water (tables 4 and 12).

EFFECT OF DIFFERENT CHEMICAL SUBSTANCES ON THE MOISTURE CONTENT OF BROWN SILT LOAM

In view of the fact that the evaporation of water from several soils treated with various chemical substances has little dependence upon the effect of these substances on the capillary water rise, it would be interesting and instructive to know whether or not a given soil, so treated, would contain more water than the untreated soil, and how this water is distributed at different depths of the soil column. King (10), for instance, has observed that, while manured and unmanured plots had practically the same amount of water in 6 feet of soil, the manured plot had considerably more water in the first and second foot than the unmanured plot. Although the solid portion of manure may have played the largest part in modifying the physical property of the soil, yet the rôle of the soluble salts in manure also might be appreciable.

In order to throw some light on this question it was decided to add some chemical substances to brown silt loam, as one of the most typical soils of the prairie region. The substances selected were: sodium chloride, as one of the least absorbed salts; potassium diphosphate, as one of the most absorbed salts; and calcium oxide, the substance often used for correcting soil acidity. Besides, its conversion (partial at least) into a carbonate form, which is most commonly used in farm practice, would take place in the soil under the conditions of this experiment.

Procedure

Galvanized iron cylinders, 18 inches long and 6 inches in diameter, were used for the purpose. They were regular evaporimeters of a large type. Around the lower end of each cylinder a double thickness of cheese-cloth was tied securely. The air-dried brown silt loam, sifted through a 1/16-inch sieve, was packed uniformly into the cylinders. Then the soil of the 3 surface inches was removed and thoroughly mixed with 19.83 gm. of sodium chloride, potassium phosphate or calcium oxide. This represents an application of one ton of the substance per acre, as the surface of the soil exposed to evaporation equals to 28.27 square inches. The soil with the applied salt was packed again into the cylinder. The cylinders were set into a proper receptacle with water, and allowed to stand in a greenhouse for 33 days.

TABLE 14

Effect of applications of chemical substances on the moisture content of brown silt loam at different depths in cylinders, water being supplied from below

DEPTH OF CYLINDER FROM SURFACE	DUPLICATE	UNTREATED			NaCl			K_2HPO_4			CaO		
		Cylinder 1	Cylinder 2	Average of four	Cylinder 1	Cylinder 2	Average of four	Cylinder 1	Cylinder 2	Average of four	Cylinder 1	Cylinder 2	Average of four
in.		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	1	35.33	35.53		36.05	34.79		37.48	38.79		38.95	39.60	
	2	35.40	36.55	35.70	35.70	34.96	35.38	37.40	38.60	38.07	38.69	39.52	39.19
2	1	36.85	37.37		40.62	39.93		39.83	40.17		41.36	41.97	
	2	36.70	37.71	37.16	40.31	39.68	40.14	39.68	39.91	39.90	40.63	42.07	41.51
3	1	37.48	39.35		39.95	39.41		39.62	40.16		41.32	41.79	
	2	37.61	37.80	38.06	40.03	39.67	39.77	39.79	40.42	40.00	40.84	41.14	41.27
4	1	38.07	39.15		39.53	39.67		38.60	38.86		38.66	38.57	
	2	37.66	37.99	38.22	39.41	39.83	39.61	38.49	38.92	38.72	38.71	39.26	38.80
5–6	1	38.69	39.65		40.06	39.64		39.36	39.42		39.29	39.03	
	2	38.68	39.37	39.10	40.00	39.26	39.74	39.11	39.60	39.35	39.12	39.25	39.17
7–8	1	40.36	41.04		39.54	38.77		38.56	38.11		39.12	39.44	
	2	40.50	41.20	40.78	39.52	38.34	39.04	38.28	38.33	38.32	39.24	39.78	39.40
9–10	1	40.50	40.50		37.51	37.29		35.67	35.91		36.63	37.33	
	2	40.78	40.37	40.46	37.68	37.32	37.45	36.20	36.23	36.00	36.61	36.75	36.83
11–14	1	40.11	39.99		39.27	38.16		37.24	37.45		36.99	37.48	
	2	39.99	40.12	40.05	38.82	37.86	38.65	37.36	37.53	37.40	36.97	38.25	37.42
15–18	1	39.04	38.79		40.05	38.66		37.58	37.52		37.60	37.79	
	2	39.25	39.39	39.41	40.28	38.45	39.36	37.51	37.51	37.53	37.75	37.99	37.78

Then different layers of soil were taken out and the moisture content was determined. The results are given in table 14 and presented graphically in figure 3. During 10 days (January 13 to 23) the amount of evaporated water from each cylinder also was determined, and the results are given in table 6.

Results and discussion

The results showing the effect of different substances were discussed in a general way with other evaporation data in the first part of this article. It might be mentioned in this connection that the results for the evaporation of the brown silt loam agree very closely with the results for the other soils studied. Studying the data in table 14 and the accompanying figure 3, one observes several features that might be of some importance. In the untreated soil the per cent of moisture gradually increased with the depth down to the 7-8 inch layer. Then it remained practically constant, or even slightly decreased. In the cylinders treated with sodium chloride the surface inch had practically the same amount of water as the surface inch in the untreated cylinders; the moisture, however, suddenly increased in the second inch to the highest per cent in the cylinder and remained so down to the 9-10 inch layer.

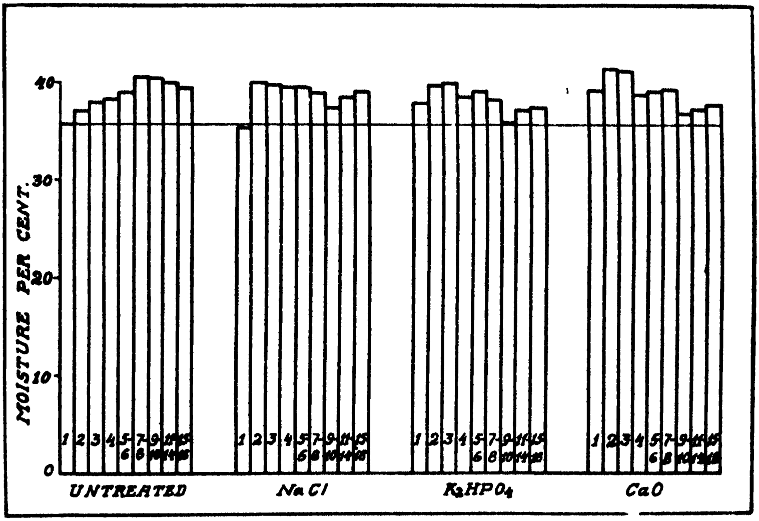

FIG. 3. MOISTURE CONTENT OF BROWN SILT LOAM AT DIFFERENT DEPTHS IN CYLINDERS AS INFLUENCED BY VARIOUS TREATMENTS

The treatment was in the first 3 inches of soil. The numbers in the columns represent the depth in inches from the surface

A somewhat similar condition was observed in the K_2HPO_4 and CaO cylinders in that the second- and third-inch layers had the highest moisture content. There were, on the other hand, some considerable differences, since in the cylinders with the latter two treatments the moisture content of the 4-8 inch depth, being practically uniform, ran somewhat lower than the moisture content at the 2-3 inch depths of the same cylinders. In all six treated cylinders there was a noticeable sudden lowering in the moisture content at the depth of 9-10 inches. This may or may not be significant.

There is still another interesting difference between soils treated with potassium monophosphate and calcium oxide, as compared with the untreated soil. The moisture content of the first inch in the treated soils was very markedly higher than that of the surface inch in the untreated soil. Again, taking the treated brown silt loam as a whole, under the conditions of this experiment, one notices that the amount of moisture in the first 8 inches from the surface is considerably greater than in the untreated soil to the same depth. In spite of this fact, however, the evaporation from the surface of the treated soil was less than in the untreated soil. The water was brought up from below and remained right beneath the surface.

It might be mentioned in this connection that in the alkali or niter spots there is found an abundance of moisture in soil right beneath its surface. The observations of Headden (9) might be of some interest to those wishing to study this problem. It is very probable, therefore, that the salts accumulated at the surface are largely responsible for the abundance of water held beneath the very thin layer of soil in alkali regions, though the warm air and high winds of the West may contribute somewhat to creating these conditions, as has been suggested by Buckingham (4).

EFFECT OF ACCUMULATED SALTS ON RATE OF WATER EVAPORATION

During the progress of the experiment it was observed that on the surface of some treated soils there would occur an accumulation of salts which were brought up with the water and deposited in the form of a crust. In the case of some salts this crust was heavier than in others. Ammonium sulfate, for instance, formed a very heavy crust, while this was practically not noticeable in the case of sodium chloride. The question might be raised whether the crust so formed would influence the rate of water evaporation. If it exerts some action, it should be noticeable in the differences in the amount of evaporated water from the treated and the untreated soils. For this reason tables 15 and 16 and figure 4 are given. They show the progress of water evaporation in several-day periods of Sassafras medium sandy loam and Elkton clay loam.

Judging from the figures in these tables and the diagram, there was no pronounced difference between the rate of evaporation at the end of the experiment and that at the beginning. The differences in water evaporation from treated and untreated soils began at once and progressed gradually and rather uniformly to the end. The curve of the heavy-crust-forming ammonium sulfate did not differ in its tendency from that of the non-crust-forming sodium chloride.

TABLE 15

Effect of different chemical substances on the evaporation of water from Sassafras medium sandy loam

Data represent grams of water evaporated from soil cylinders at different periods; average of two determinations taken

TREATMENT	DAYS									
	4	8	13	17	22	27	32	36	40	48
	gm.	gm	gm	gm.	gm	gm.	gm	gm.	gm.	gm.
Untreated.	52	99	149	193	245	298	358	424	[illegible]	626
$(NH_4)_2SO_4$..	35	71	105	137	176	215	269	327	380	480
NaCl....	27	55	85	113	141	174	223	270	312	393
KH_2PO_4....	43	85	128	167	210	254	316	383	450	580
CaO	49	93	141	183	229	275	333	399	466	604

TABLE 16

Effect of different chemical substances on evaporation of water from Elkton clay loam

Data represent grams of water evaporated from soil cylinders at different periods; average of two determinations taken

TREATMENT	DAYS						
	5	10	15	21	25	30	35
	gm	gm.	gm	gm	gm	gm.	gm
Untreated	129	231	382	542	648	819	900
$(NH_4)_2SO_4$	85	149	239	344	415	432	590
NaCl ..	58	102	168	237	279	353	384
KH_2PO_4 .	95	167	261	356	415	511	557
CaO..	36	66	110	171	204	272	313

EFFECT OF TEXTURE OF SOIL ON THE RATE OF EVAPORATION OF WATER

As recorded in the description of the procedure in conducting the experiments on the effect of chemical substances on the evaporation of soil water, a Livingston's (15) atmometer cup was used with the evaporimeters. This atmometer being the same throughout the experiment, it afforded an opportunity to study the effect of the texture of the soil on the rate of water evaporation, when water was supplied 12 inches below the surface. Table 17 gives the amount of water evaporated from the untreated cylinder and from the atmometer for each soil studied, and also the ratio of the first to the latter.

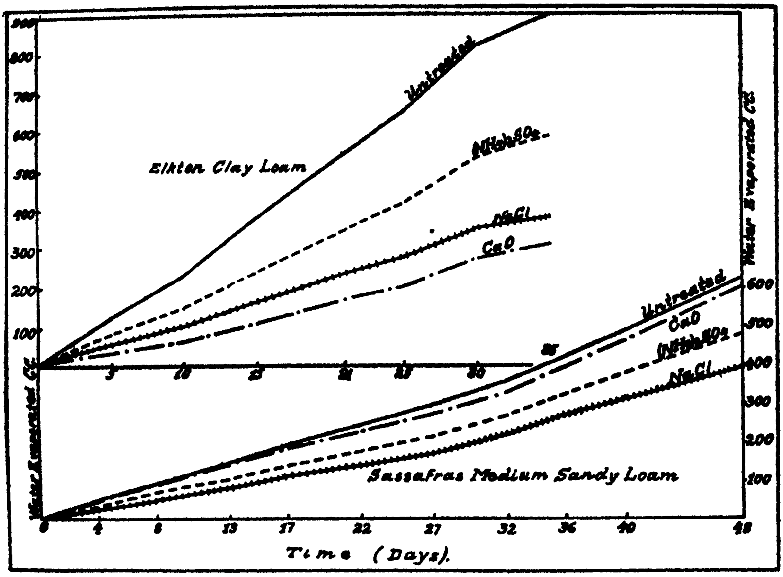

FIG. 4. PROGRESS OF EVAPORATION OF WATER FROM ELKTON CLAY LOAM AND SASSAFRAS MEDIUM SANDY LOAM AS INFLUENCED BY VARIOUS TREATMENTS

Note the position of the CaO curve in respect to those of the two salts in the different types of soil.

TABLE 17

Influence of texture of the soil on the evaporation of water from soil cylinders 12 inches in depth, water being supplied from below

SOIL	WATER EVAPORATED FROM UNTREATED CYLINDER (A)	WATER EVAPORATED FROM ATMOMETER (B)	RATIO A B
	gm	*gm*	
Sea sand	334	656	0 509
Sassafras medium sandy loam	626	708	0 884
Penn loam	960	697	1 377
Elkton clay loam	900	590	1 525
Muck	957	567	1 688

This ratio was the smallest for the sea sand, and gradually increased with the increase in the fineness of the soil Thus, for mineral soils the ratio was greatest in the case of Elkton clay loam. In other words, when water was supplied at 12 inches below the surface of the soil, the clay soil evaporated water most rapidly, and the sand most slowly. Muck, being composed largely of organic material, evaporated water even more rapidly than the Elkton clay loam.

PRACTICAL DEDUCTIONS

Besides the theoretical considerations involved in the interpretation of these results, there might be mentioned an important practical deduction that could be made with considerable safety. Although it is always desirable and highly advisable to check all laboratory and greenhouse pot and cylinder experiments by the more elaborate field experiments, the former often serve as an indicator (though qualitative) as to what would happen in the field under similar treatment. These deductions are still more trustworthy if the greenhouse experimental results correlate with the practical field observations. For this reason the results presented above have considerable practical significance.

Several soluble salts or lime, when applied to some typical agricultural soils, do exert a very pronounced influence on the rate of water evaporation and also on the amount of water present at or near the surface of the soil. From practical field observation, in alkali regions, we do not know whether or not the salts decrease the evaporation of water, but we do know that under those conditions an abundance of water is held at the surface. Since the studied soluble salts are not able to hasten the capillary rise of water in soils to an appreciable extent (sodium chloride diminished it considerably), it is reasonable to conclude that even the field observations bear out the point that soluble salts in soil do decrease the evaporation of water. This will help us to understand why alkali soils are so wet directly beneath the surface.

Though these experiments were carried on in the greenhouse, it is safe to suppose that an application of commercial fertilizers to soils under field conditions would tend to help in the conservation of soil moisture through a diminished evaporation. Besides, it would help to bring the water to the surface and thus make it more available for the growing crop. This would be especially valuable at the time of the germination of seed, for which a right amount of moisture, besides the right temperature, might be the most important factor.

With barley, for instance, it was found by the author (19) that with several agricultural soils there must be present a considerable amount of water before the germination would take place. This being the case, it would seem that a fair amount of soluble salts at the surface of soil would help to make the conditions more favorable for seed germination and an early growth. This contention is well corroborated by a fairly common practice in some sections of the country to apply some readily available fertilizer at the time of seeding.

It is true that the application of salts in these experiments were too excessive to be practiced in actual farm operations. It stands to reason, however, that some action of various salts would be felt when applied in smaller amounts. The experiment performed with various amounts of ammonium sulfate on Penn loam in the same cylinders, as described in the first part of the article, strengthens this contention. The results are presented in table 18 and show that even the smallest application exerted a pronounced influence on the evaporation of soil moisture. This application, 250 pounds per acre, lies within the range of actual farm practice.

So far as the results with lime are concerned, they represent an amount frequently applied to soils to correct the soil reaction. Indeed, much higher applications of lime sometimes are recommended and made. Thus, it would seem that the effect of lime on evaporation and on the distribution of water in some agricultural soils might be very considerable. This would be especially true in heavier types of soil and, indeed, it is on this type of soil that lime is most advantageous, as observed in farm practice.

TABLE 18

Effect of different amounts of ammonium sulfate on evaporation of water from Penn loam

Duration 33 days

TREATMENT	CYLINDER 1	CYLINDER 2	AVERAGE	DECREASE OVER UNTREATED	WATER SAVED BY TREATMENT
	gm.	*gm.*	*gm.*	*gm.*	*per cent*
Untreated	700	740	720		
250 lbs. $(NH_4)_2SO_4$	660	668	664	56	7.8
500 lbs. $(NH_4)_2SO_4$	625	634	635	85	11.8
1000 lbs. $(NH_4)_2SO_4$	598	624	611	109	15.1
2000 lbs. $(NH_4)_2SO_4$	560	570	565	155	21.5

SUMMARY

The experimental work recorded in this paper was performed with ammonium sulfate, potassium sulfate, magnesium sulfate, sodium chloride, calcium nitrate, potassium phosphate and calcium oxide, with which some agricultural soils were treated in order to ascertain the influence these salts and the base would have on the evaporation, capillary rise and eventual distribution of water in soil.

The indications are that the following conclusions might be drawn from the results obtained:

1. Soluble salts materially decrease the evaporation of soil moisture.

2. There is a direct dependence of the efficiency of salts in decreasing the water evaporation upon the osmotic concentration of the soil solution in the surface inch of soil. With few exceptions the results tend to show that the greater the osmotic concentration of soil solution in the first inch of soil the greater is the depresson of the evaporation of moisture.

3. For the soluble salts studied sodium chloride and calcium nitrate were most effective in checking the evaporation of water, and potassium sulfate and potassium phosphate were least effective, while ammonium sulfate and magnesium sulfate occupied an intermediate position.

4. Calcium oxide in this respect was least effective in sea sand and sandy loam and most effective in clay loam, as compared with the soluble salts studied.

5. With two agricultural soils sodium chloride decreases the capillary rise of water. Calcium oxide in drab clay and potassium phosphate in brown silt loam show a tendency to accelerate the water rise. Ammonium sulfate shows no pronounced effect, when applied in the same amount.

6. Sodium chloride, potassium phosphate and calcium oxide very materially influence the distribution of moisture in brown silt loam. There is a very pronounced tendency for treated soil to contain more water in the first 8 inches, as compared with the untreated soil.

7. Soil treated with potassium phosphate or calcium oxide even in the surface inch contained more water than the untreated soil, and yet evaporation from treated soil was decreased.

8. Comparing the untreated soils, when water was supplied at 12 inches below the surface, the extent of evaporation depended on the texture of the soil. With the increase in the amount of fine material in the soil the evaporation increased.

The work recorded in this article was partly done at the New Jersey Agricultural Experiment Station while the author was a graduate student at Rutgers College. To Dr. J. G. Lipman, under whose supervision the work was started, the author is indebted for his interest in the work and for many helpful suggestions. The work was finished at the Illinois Agricultural Experiment Station, and author is very grateful to Prof. J. G. Mosier and Dr. R. S. Smith for the use of the laboratory equipment, and to Dr. R. Stewart for critically reading the manuscript.

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THE EFFECT OF GYPSUM ON BACTERIAL ACTIVITIES IN SOILS[1]

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INTRODUCTION

The discovery of gypsum and its fertilizing value was made at about the same time in Germany and in France in the latter part of the eighteenth century. The use of gypsum spread quickly to other countries, first to America and considerably later to Great Britain. Just how it acts is little understood, but the two explanations which have been given are that it supplies the sulfur needed for plant growth, or that it acts as a corrective agent by favoring beneficial groups of bacteria, while the development of injurious species is prevented. Recently, some authors have secured results which indicate that sulfates act on complex compounds like the silicates liberating potassium and phosphorus. In other words, there are indications that sulfates may render the essential plant-food constituents available for the use of plants.

The need of sulfur as a plant-food is evident from the fact that the proteins of plants contain sulfur, and there is no question but that plants utilize sulfur in the form of sulfates just as they utilize phosphorus as phosphates and and nitrogen as nitrates.

Sulfur has been supposed to be present in soils in sufficient amounts to keep all crops supplied. Recently, however, the possibility of value from using sulfur as a fertilizer has been indicated.

Many comparatively recent analyses made at Rothamsted and elsewhere have shown that certain soils are deficient in sulfur and that there is a rather constant ratio between phosphorous and sulfur. Soils that are low in phosphorus and need phosphorus fertilizers may therefore respond also to sulfur fertilizers. The application of phosphorus in the form of acid phosphate supplies sulfur as calcium sulfate along with phosphorus and when this material is used it may be possible to insure an ample supply of sulfur to meet the requirements of crops.

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There are certain soils, especially in the Middle West, which are fairly well supplied with sulfur and probably would not respond to sulfur fertilization at the present time. But the fact that the content of sulfur is gradually being exhausted in some soils should not be lost sight of. Exeriments at Rothamsted showed that the average annual loss of sulfur by drainage amounted to 50 pounds per acre and the amount of sulfur trioxide precipitated with the rain was found to be about 15 to 20 pounds per acre per annum. The conclusions reached were, therefore, that the losses of sulfur from the soil by drainage and cropping are much larger than can be met by the amounts brought down by rain and that some carrier of sulfur such as farm manure, acid phosphate, ammonium sulfate, sulfate of potassium, or gypsum must be applied to soils, if they are to be maintained in a permanently fertile condition. Of late, the use of gypsum as a fertilizer has been tested by a few of the agricultural experiment stations in the United States and it is being recommended in some sections of the country as a profitable fertilizing material. Extensive experiments should be carried out in the field, however, before the use is recommended under any particular conditions. Tests are now under way in Iowa to determine its value and in other states there is a considerable interest manifested in gypsum. It may be that the material will play an important rôle in many states in maintaining fertility.

HISTORICAL

Before entering upon the discussion of the experimental data obtained in this work, a brief history of the results secured by previous investigators may be given. Comparatively few studies have been made of the effects of gypsum on bacterial activities but some tests of its influence on ammonification, nitrification and azofication have been carried out with interesting results.

The effect of gypsum on bacterial activities

Severin (44) in laboratory tests with sterilized and unsterilized manure (inoculating in the latter case with pure cultures of organisms capable of inducing ammoniacal fermentation as well as with water extracts of manure), found that the addition of 4 per cent gyspum to the manure intensified the decomposition of the manure 10 to 20 per cent and at the same time prevented any loss of ammoniacal nitrogen.

Heinrich (19) found, likewise, that gypsum was a very effective preservative of manure. C. B. Lipman (22) concluded from his work that gypsum stimulated the beneficial soil organisms on the roots of leguminous plants.

J. G. Lipman (25) tested the influence of gypsum on the number of soil bacteria and concluded that applications of gypsum were not injurious to soil bacteria, nor to the plants themselves. In fact he observed from the data secured that the amounts of nitrate nitrogen expressed as parts per million were favorably affected by the addition of gypsum. Lipman and his associates (28) found that the addition of gypsum to a soil often increased the total nitrogen of the crop removed from the soil. The same authors (27) experimenting on the availability of nitrogenous materials as measured by ammonification, demonstrated that the so-called stimulants—potassium iodide, and copper, zinc, manganese, ferrous and calcium sulfates—gave varying and inconclusive results, while phosphates appeared to favor ammonification. Lipman found from box experiments that the nitrogen content of soybeans was distinctly increased by the use of lime, while gypsum appeared to have no effect. Neither lime nor gypsum showed any appreciable effect on buckwheat.

Dezani (10) in his experiments showed that no material increase in nitrification was obtained when gypsum was added in amounts varying from 0.5 to 2 per cent. Lemmerman and Fresenius (21) found that the addition of calcium carbonate to soils in pots to the extent of 1 per cent reduced the volatilization of ammonium carbonate and increased the absorptive power of the soil for ammonia. Caustic lime had the opposite effect. Calcium sulfate and calcium chloride reduced the loss solely by their direct action on the ammonium carbonate. Patterson and Scott (38) studied the influence of caustic lime, calcium carbonate, gypsum, ferric hydrate, sodium chloride, citric acid, starch, sugar and acid phosphate on nitrification in soils and found that caustic lime practically stopped all nitrification. Calcium carbonate was the most efficient of the substances tested in stimulating the process and gypsum had little effect on nitrification. Nishimura (2) reported that gypsum and Kainit were found to be less effective than acid phosphate in fixing ammonia.

Fred and Hart (15), found that the sulfates of calcium and potassium increased ammonification to a small extent and calcium sulfate gave a slight increase in carbon-dioxide production. They further claimed that sulfates, although as low in amount in most soils as phosphates, would not in all probability have the same effect on the crop-producing power of soils as phosphates, and they attribute the inequality to the differences in the effect of the two acid radicals on the soil.

Peck (39) studied ammonification, nitrification, denitrification and azofication with three samples of soils by inoculating nutrient solutions with small amounts of the soil, or by observing the progress of nitrogen transformation in the soils themselves. He found that the addition of lime as carbonate, sulfate or phosphate stimulated ammonification; as regards nitrification calcium carbonate exerted the most favorable action, while gypsum had no effect. Sugar showed an increased azofication. Hart and Tottingham (18) in their work showed that sulfates as compared with soluble phosphates had very little effect on the soil flora.

Pitz (41) in his work with sulfur and calcium sulfate concluded that the addition of calcium sulfate to the soil had no marked effect on the total number of bacteria growing on ordinary agar plates, nor did it produce any marked increase in ammonification or nitrification. The material was found, however, to stimulate the growth of pure cultures of red clover bacteria and also to increase the root development of red clover in nutrient solution and in soil extract. In small amounts it was also found to increase the yield of red clover and the number of nodules.

Brown and Johnson (7) found that calcium sulfate in ordinary applications had no detrimental effect on sulfofication, but very large applications might decrease the rate of oxidation of sulfur.

The effect of gypsum on crops

The effect of gypsum on crops has been studied to some extent and the influence on legumes noted especially. Hart and Peterson (17) determined the sulfur content of a number of common farm crops and in agreement with other investigators they showed that the quantity is much higher than that found by Wolff in the ash from such products. Withycombe (50) reported the yields of green clover from plots receiving different quantities of gypsum and showed a gain of 22 per cent in favor of gypsum. Dymond and his associates (13) from their investigations conclude as follows:

> There is not sufficient sulfuric acid in the soil or supplied by rain for heavy-yielding crops rich in albuminoid, either for the production of the greatest yield or the highest feeding value, and for such crops a sulfate should be included in the artificial manure. For cereal crops and for permanent pastures, the soil and the rain provide all the sulfuric acid necessary.

Takeuchi (49) carried on pot experiments with peas, beans, oats, rice and spinach in which gypsum was used in connection with ammonium sulfate, sodium nitrate, potassium sulfate and different forms of phosphorus. He observed that as a rule gypsum decreased the yield when used in connection with acidic fertilizers and increased the yield when used with basic fertilizers. He found further, that gypsum exerted a favorable influence in overcoming the injurious effects of magnesium.

Dusserre (12) found that the application of gypsum greatly increased the yields of potatoes and beets and in the case of potatoes, the mineral matter and starch were also considerably increased by the use of gypsum. Aston (3) reported that gypsum was the most effective lime compound in increasing the yield of cruciferous plants on soils containing an excess of magnesia, but ground limestone was the most effective in increasing the yields of oats and grasses. Meyer (31) found that calcium and magnesium carbonates had a beneficial effect on the yield of red clover, mustard and potatoes on acid soils, whereas gypsum reduced the yield. Rusche (42) demonstrated that magnesium and calcium sulfate generally had a good effect on the germination of barley, beans, beets, alsike clover, red clover, white clover, wheat and other seeds.

Patterson (37) found that the application of raw-rock phosphate gave better results than gypsum in a rotation of corn, wheat, timothy and clover on a sandy soil. Shedd (45) showed that the addition of sulfur or gypsum to a fertilizer containing only nitrogen, potassium and phosphorus, when applied to a Graves County soil, gave a decided increase in growth of Yellow Pryor tobacco over the fertilizer alone. G. O. Brown (6) in his experiment with calcium sulfate found that an application of air-slaked lime, followed by a 100-pound application of land plaster after the first cutting of alfalfa, resulted in a greatly increased vigor and a total yield considerably in excess of the untreated crop.

The most recent investigation along this line was reported by Miller (32) who worked with three Oregon soils. The first was taken mainly for its high sulfur content, the second one because it did not respond to sulfur treatment in the field, while the third did respond to elementary sulfur fertilizer. The highest sulfur content was 0.183 per cent and the lowest 0.02 per cent. The results show that the addition of calcium sulfate and elementary sulfur enhanced the growth of rape, oats and clover grown in pots in the greenhouse. The great increase in the nitrogen content of the clover grown on the soil where sulfates had been added was the result in all probability of the stimulating action of the sulfate on the legume bacteria. The sulfate increased the root development and the number of nodules on the clover grown in the soil in pots. Tacke (48) explained in his work that the injurious effect of gypsum observed especially in the case of leguminous plants was due to the setting free of acids to which such plants are especially sensitive.

Parshad (36) in his work with gypsum, found that it proved a valuable fertilizer on indigo and that top-dressing was the least beneficial method of applying it. Katayama (20) in experiments carried on at Tokyo, showed that rice yielded better and had a better color when grown on land manured with gypsum. C. B. Lipman and Gericke (23) found that calcium sulfate in varying quantities strongly antagonized the sodium sulfate in clay adobe soil where barley was grown.

The effect of gypsum on available plant-food

Several experiments have indicated an effect of gypsum on the solubility of plant-food.

Storer (46) showed a greater amount of phosphorus in clover taken from land manured with gypsum. Pfeffer (40) states that Knop found that where seeds were grown in water containing calcium sulfate, the calcium of the salt was absorbed in a somewhat greater amount than the acid. If this is true, it is easy to see how calcium sulfate can assist in the assimilation of phosphorus even though the phosphates are found to be less soluble in a calcium sulfate solution.

Dusserre (11) found that the most effective agents in rendering soil potash soluble in distilled water were gypsum and sulfate of ammonia. Dumont (8), studying the effect of gypsum upon both granitic soils and the separates from these obtained by mechanical analysis, found that when mixed with about one-third its weight of gypsum, moistened and allowed to stand, the soil gave increasing amounts of water-soluble potash with lengthening periods of contact between soil and gypsum.

Schreiber (43) reported experiments which indicated that gypsum had a marked though limited effect in setting free the potash of the soil. Morse and Curry (34) found that lime and gypsum in contact with feldspar increased the solubility of potassium. Soane (47) in his general discussion of a series of pot experiments to determine the chemical effect of the application of gypsum on soils, reported that the effect of gypsum alone was insignificant. When combined with soluble potash, however, it seemed to produce a beneficial effect.

Morse and Curry (35) found that when powdered feldspar was treated with gypsum the solubility of potash in water was increased. Bradley (4) found that gypsum added both to soils from western Oregon and to the mineral pegmatite markedly increased the content of water-soluble potash. Andre (1) observed a greatly increased solubility of the potash of microline when this was treated with gypsum. C. B. Lipman (24) concluded that calcium sulfate is the most powerful soil stimulant we have and that its effect is due mainly to its liberation of plant-food, especially potassium.

McMillen (30) found that when various soils, mixed with 1 per cent of gypsum, were kept for 3 months under moisture conditions similar to those prevailing in the field there were marked increases in the content of water-soluble potash. It is suggested, that in experiments previously reported by various investigators where gypsum was not found to bring about such an increase the lack of any action might be due to the fact that the condition of contact between the soil and gypsum was not the same as would occur in the field.

Fraps (14) found that the addition of sulfate of lime, nitrate of soda, or other salts, has no such effect upon rendering potash available to plants as has been claimed. Briggs and Breazeale (5) reported that gypsum solutions depressed the solubility of the potassium in orthoclase, the quantity of potash in solution decreasing progressively as the concentration of the calcium sulfate increased. McCool (29) found very recently that the effect of the calcium sulfate on the rate of the formation of soluble potash was negligible in the first period of 2 days and slightly reduced it in the second period of 17 days. He claims that soils of different texture and composition probably would be affected somewhat differently.

Greaves and Carter (16) in their recent investigation also reported that the strong stimulant sodium chloride to a great extent acts by rendering phosphorus soluble, whereas the equally strong stimulant calcium sulfate acts by rendering more nitrogen available.

EXPERIMENTAL

Dumont (9), summarizing in his paper the theories advanced regarding the value of gypsum when applied to the soil, points out that it serves as a stimulant, that it serves as a plant-food, that it acts as an absorbent for volatile compounds like ammonia, and that it helps in making plant-food available.

The work presented in the subsequent pages was planned to throw some light on the following questions:

(a) Does calcium sulfate favor the activities of desirable organisms of the soil?

(b) Does it serve as a plant-food?

(c) Does it make certain plant-food constituents more available?

Plan of the experiments

The soil used in these experiments was secured from an orchard at Ames and is classified as Miami silt loam. The chemical analysis is given in table 1.

TABLE 1

Soil analysis

Total sulfur	0.168 per cent
Total sulfates	0.005 per cent
Total potassium	1.410 per cent
Water-soluble potassium	0.0027 per cent
Limestone requirement	2500 pounds per acre

Forty-eight pots were each filled with 40 pounds of soil which had been sifted through a coarse sieve, so as to remove the roots and pebbles. The treatment of these pots was as follows:

1, 2	Check
3, 4	Limestone to neutralize acidity plus 2 tons (3¼ tons)
5, 6	100 pounds $CaSO_4$ per acre
7, 8	500 pounds $CaSO_4$ per acre
9, 10	1000 pounds $CaSO_4$ per acre
11, 12	100 pounds $CaSO_4$ per acre plus limestone (3¼ tons)
13, 14	500 pounds $CaSO_4$ per acre plus limestone (3¼ tons)
15, 16	1000 pounds $CaSO_4$ per acre plus limestone (3¼ tons)

The first set of these pots from 1 to 16 were kept fallow, the second set of 16 pots similarly treated were seeded to wheat, and the third set of 16 pots with similar treatment as the first two sets, were seeded to red clover. The pots were kept in the greenhouse under uniform temperature conditions. The moisture content of the soils in the pots was kept up to 15 per cent of the dry weight of the soil.

After the seeds had germinated in the pots, the plants of red clover and wheat were thinned so as to leave only 6 plants in each pot.

BACTERIOLOGICAL

In order to ascertain whether gypsum favors the activities of beneficial soil organisms it was planned:

1. To study the action of gypsum on bacterial activities as measured by ammonification, nitrification and azofication.
2. To study the action of gypsum in soil and in solutions inoculated with pure cultures of *Bacillus radicicola* isolated from alfalfa, Canada field peas, red clover and soybeans.

The first samples were drawn 15 days after the pots were filled. The surface soil was removed to a depth of five inches and the samples drawn with a sterile spatula. The surface soil was then replaced in the various pots, the samples were brought to the laboratory, and the moisture content, total nitrogen and nitrates determined in each case.

A. Ammonification

One hundred grams of each soil were put in duplicate tumblers and 5 gm. of dried blood added to each and stirred in thoroughly. The moisture content was brought up to the optimum, allowing 12 cc. for the dried blood used. The tumblers were incubated at room temperature for a period of 7 days. Ammonia was determined by the aeration method, potassium carbonate being used.

B. Nitrification

One-hundred-gram quantities of each soil were weighed out in duplicate tumblers and 100 mgm. of ammonium sulfate in solution added. The moisture content was adjusted to the optimum. The tumblers were covered and incubated for 5 weeks at room temperature, the moisture content being adjusted to the optimum every seventh day. The nitrates present at the end of the 5-week period were determined by the phenol-disulfonic acid method.

C. Azofication

One-hundred-gram quantities of soil were weighed out in duplicate tumblers and 5 gm. of dextrose added to each and thoroughly mixed in. The moisture content was made up to the optimum and the soils incubated at room temperature for a period of 10 days. At the end of that time the total nitrogen present was determined in duplicate by the Kjeldahl method, and the nitrogen content at the beginning subtracted from this gave the amount of nitrogen fixed.

D. The effect of gypsum on radicicola

In order to study the effect of gypsum on the azofying power of pure cultures of *B. radicicola* from alfalfa, field peas, red clover and soybeans, the following method was employed:

One-hundred-gram quantities of soil with various additions of gypsum were put into tumblers. One set of these tumblers was sterilized and the other was not. Both sets of tumblers were inoculated with pure cultures of *B. radicicola* from red clover. After incubating for a period of 10 days the amount of nitrogen fixed was determined. Other sets of tumblers were arranged as previously and inoculated, respectively, with pure cultures of *B. radicicola* from alfalfa, Canada field peas, red clover and soybeans in unsterilized soil alone. In each case the nitrogen fixed was determined.

One hundred cc. of "radicicola solution"[3] was put into 500-cc. flasks, various amounts of gypsum added, sterilized and inoculated with pure cultures of *B. radicicola* from different legumes The nitrogen fixed in each case was determined by duplicate analyses.

Crop tests

After the plants in the pots had grown to maturity, the dry weight of straw and seed together was determined.

Plant-food tests

The acidity of the soil after the crops were harvested was determined by the modified Tacke method and reported in terms of tons of limestone required per acre.

In order to test the solubility of potash and the increase of the nitrogen content in the soil and in the crop, an experiment was arranged in the greenhouse. Sixty-four pots, each containing 10 pounds of Miami silt loam soil, were prepared and the same applications of gypsum and lime as in the previous experiment were made. These pots were sown to alfalfa, field peas, red clover and soybeans. Just before sowing, the seeds were inoculated with pure cultures of *B. radicicola*. The moisture content of the pots was kept up to 15 per cent. At the end of the experiment the crops were harvested, dried and weighed. They were ground finely and determinations of total nitrogen and potassium were made in duplicates. The total nitrogen content of the soil was determined by the usual Kjeldahl method, $CuSO_4$ and K_2SO_4 being used for the digestion with H_2SO_4, with the aeration method of distillation.

In order to determine the water-soluble potassium, 100 gm. of soil was shaken with 200 cc. of distilled water for 4 hours in a mechanical shaker. The clear, supernatant liquid was filtered and aliquot portions of the filtrate were taken to determine the soluble potassium. The fusion for total potassium was accomplished according to the method of J. Laurence Smith for total alkali. Potassium was precipitated as the platinic chloride without previous removal of calcium, which was subsequently removed by washing with acidulated alcohol (33). Potassium was weighed as potassium platinic chloride in the case of the water-soluble as well as in the case of total potassium.

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[3] Radicicola solution—

Dissolve in 1000 cc. of tap water,
10 gm. saccharose
1 gm. K_2HPO_4

Stir until dissolved. Neutralize, using phenolphthalein as indicator. Sterilize in the autoclave at 15 pounds pressure for 15 minutes.

THE BACTERIOLOGICAL RESULTS

Ammonification, nitrification and azofication

The results of the tests of ammonification, nitrification and azofication at the first sampling are given in table 2.

TABLE 2

Effect of $CaSO_4$ *on ammonification, nitrification and azofication—Sampling 1*

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	AMMONIFICATION		NITRIFICATION		AZOFICATION	
		NH_3 nitrogen in 100 gm. of air-dry soil	Average	NO_3 nitrogen in 100 gm of air-dry soil	Average	Nitrogen fixed in 100 gm of air-dry soil	Average
		mgm.	*mgm*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None	130 7		10 60		1 50	
2	None	130.9	130 80	10 40	10 50	1.50	1.50
3	3½ tons $CaCO_3$	150 7		16 56		4.50	
4	3½ tons $CaCO_3$	150 0	150 35	16 56	16 56	4 50	4 50
5	100 pounds $CaSO_4$	126 0		8.90		6.00	
6	100 pounds $CaSO_4$	123 9	124 95	8 90	8 90	6.50	6 25
7	500 pounds $CaSO_4$	129 1		9.30		4.00	
8	500 pounds $CaSO_4$	130 4	129.75	9 30	9 30	4.00	4.00
9	1000 pounds $CaSO_4$	62 6		10.55		4 00	
10	1000 pounds $CaSO_4$	63.2	62 90	10 33	10 44	4.25	4.12
11	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	72.4		17.41		10.00	
12	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	71 3	71 85	17.20	17 30	11.50	10 75
13	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	73 2		20 65		10.50	
14	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	72.2	72 70	20 65	20 65	10 60	10 55
15	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	66.9		20 60		12.00	
16	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	66.3	66 60	20 60	20 60	12.50	12.25

Examining this table it is evident that the ammonification of dried blood was reduced by all the applications of gypsum, the greatest reduction occurring where the gypsum was applied at the rate of 1000 pounds per acre. The application of lime with the gypsum did not increase ammonification but, on the other hand, decreased it considerably. This effect of lime was hardly expected since the application of lime alone greatly stimulated ammonification.

Nitrification also was slightly reduced in the soils which received the various amounts of gypsum alone, but the process was increased considerably where lime alone, or lime with gypsum was used, the gypsum and lime together showing a greater increase than the lime alone. This increase in nitrification from the use of lime bears out previous results which have shown that the nitrifying organisms are very sensitive to a lack of lime. The beneficial effect of gypsum with lime indicates that the effect of the material on nitrification may be changed from detrimental to desirable by applying it with lime.

There was a marked increase in the azofying power of the soil when gypsum alone was applied, the smallest application showing the greatest effect. When lime was added with the gypsum a still greater effect on azofication was evidenced, the largest amount in this case giving the greatest effect. Lime showed about the same influence as gypsum alone and the large increase with the two materials was evidently due to the gypsum. As in the case of nitrification, gypsum seemed to exert its largest influence on azofication when the acidity of the soil was neutralized with lime.

The results secured at the second sampling appear in table 3.

TABLE 3

Effect of $CaSO_4$ *on ammonification, nitrification and azofication—Sampling 2*

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	AMMONIFICATION		NITRIFICATION		AZOFICATION	
		NH_3 nitrogen in 100 gm. of air-dry soil	Average	NO_3 nitrogen in 100 gm. of air-dry soil	Average	Nitrogen fixed in 100 gm. of air-dry soil	Average
		mgm.	*mgm.*	*mgm.*	*mgm*	*mgm.*	*mgm.*
1	None	95 90		13 9		1.20	
2	None.. .	97 30	96 60	13 9	13 90	1 20	1 20
3	3½ tons $CaCO_3$. . .	136 95		16 1		1 70	
4	3½ tons $CaCO_3$. .	139 81	138 38	15 3	15.70	1.70	1.70
5	100 pounds $CaSO_4$	84 15		12 2		4 70	
6	100 pounds $CaSO_4$.	94 71	89 43	12 2	12 20	4 30	4 50
7	500 pounds $CaSO_4$	103 07		13 3		4.80	
8	500 pounds $CaSO_4$.	101 64	102 35	13 3	13 30	4 80	4.80
9	1000 pounds $CaSO_4$.	113 19		16 1		6 30	
10	1000 pounds $CaSO_4$ .. .	114 07	113 63	16 7	16 40	6 30	6 30
11	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	153 35		17 2		9.55	
12	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$ ..	155 22	154 28	18 2	17 70	7.05	8.30
13	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$.	118.47		17 3		4 30	
14	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$. .	113.08	115 77	17 3	17.30	4 30	4.30
15	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$.	135 30		12 1		2.80	
16	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$. .	136 40	135 85	12 4	12.25	2 80	2.80

Examining this table it is found that the ammonification of dried blood was not affected by the small amounts of gypsum but the larger applications gave an increase which was quite pronounced with the largest amount. When lime was used with the gypsum, still larger increases were obtained, the smallest amount of gypsum giving the greatest effect in this case. Lime alone brought about an increase just as it did in the first instance, and only in one case did the gypsum with lime show an increase over the lime alone.

Nitrification was increased in the soil that had received gypsum at the rate of 1000 pounds per acre. Smaller applications of the gypsum than this had no effect on the nitrifying organisms. Lime alone stimulated nitrification, and gypsum with lime gave a still greater stimulation on the activities of nitrifying organisms, except where the largest application of gypsum was made, in which case no effect of either material was noted.

Azofication was increased where gypsum alone was applied, the greatest increase occurring with the application of the largest amount of gypsum. Lime alone had a slight effect and gypsum with lime showed a greater effect than gypsum alone only with the 100-pound application. With the larger amounts the activities of azofying organisms were reduced.

It may be noted in this table that gypsum applied alone at the rate of 1000 pounds per acre increased the activities of the azofying and the nitrifying organisms, but when an application of lime was made with it these processes were decreased. On the other hand, lime applied with gypsum at the rate of 1000 pounds increased ammonification more than did the gypsum alone.

The results obtained at the third sampling are given in table 4.

TABLE 4

Effect of $CaSO_4$ *on ammonification, nitrification and azofication—Sampling 3*

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	AMMONIFICATION		NITRIFICATION		AZOFICATION	
		NH_3 nitrogen in 100 gm. of air-dry soil	Average	NO_3 nitrogen in 100 gm. of air-dry soil	Average	Nitrogen fixed in 100 gm. of air-dry soil	Average
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None	132.7		18.0		2.75	
2	None	134.1	133.40	18.4	18.20	2.75	2.75
3	3½ tons $CaCO_3$	167.5		24.5		2.25	
4	3½ tons $CaCO_3$	158.2	162.85	24.5	24.50	2.25	2.25
5	100 pounds $CaSO_4$	130.4		20.6		4.75	
6	100 pounds $CaSO_4$	131.3	130.85	20.7	20.65	4.75	4.75
7	500 pounds $CaSO_4$	129.4		14.4		1.75	
8	500 pounds $CaSO_4$	127.1	128.25	14.4	14.40	1.75	1.75
9	1000 pounds $CaSO_4$	135.1		15.3		2.25	
10	1000 pounds $CaSO_4$	135.8	135.45	15.3	15.30	2.25	2.25
11	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	141.0		16.7		3.75	
12	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	142.2	141.60	17.3	17.00	3.25	3.50
13	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	148.8		16.2		3.00	
14	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	147.9	148.35	16.2	16.20	3.75	3.37
15	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	158.1		15.5		9.75	
16	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	158.5	158.30	15.6	15.55	9.75	9.75

The gypsum applied at the rate of 1000 pounds per acre showed a slight increase in the ammonification of dried blood while the smaller applications had no effect. Lime alone increased ammonification and when the two materials were used together, the ammonifying power of the soil was increased but the increase was smaller than when the lime was applied alone.

At this sampling, gypsum did not have any pronounced effect on nitrification except when applied at the rate of 100 pounds per acre, when a slight increase in nitrification occurred. Lime alone increased nitrification but when used with gypsum there was no effect evidenced on the process, the nitrifying power being actually lower than in the check soil.

There was a marked increase in the azofying power of the soil when gypsum was applied at the rate of 100 pounds per acre, but the larger applications had no effect whatever.

Lime alone had no effect on this process but, when used with gypsum increases were obtained in every case, the greatest increase occurring when the gypsum was applied at the rate of 1000 pounds per acre.

The results of the tests of ammonification, nitrification and azofication at the fourth sampling are given in table 5.

TABLE 5

Effect of $CaSO_4$ *on ammonification, nitrification and azofication—Sampling 4*

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	AMMONIFICATION		NITRIFICATION		AZOFICATION	
		NH_3 nitrogen in 100 gm. of air-dry soil	Average	NO_3 nitrogen in 100 gm. of air-dry soil	Average	Nitrogen fixed in 100 gm. of air-dry soil	Average
		mgm	*mgm*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None	126 85		14.67		2.90	
2	None	125 67	126 26	14.67	14.67	2 90	2.90
3	3½ tons $CaCO_3$	152 45		21.84		5 60	
4	3½ tons $CaCO_3$	151 15	151 80	21 87	21.87	5.60	5.60
5	100 pounds $CaSO_4$	121 42		12.27		10.60	
6	100 pounds $CaSO_4$	119 88	120 65	12.27	12 27	8.60	9 60
7	500 pounds $CaSO_4$	123 78		12.45		2.60	
8	500 pounds $CaSO_4$	124 84	124 31	12 30	12.37	2 60	2.60
9	1000 pounds $CaSO_4$	116 58		9 05		2.40	
10	1000 pounds $CaSO_4$	111.94	114 26	9 05	9.05	2.90	2.65
11	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	128 62		13.10		6 10	
12	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	129.47	129.04	12 84	12 97	6.60	6.35
13	500 pounds $CaSO_4$ + 3½ tons lime	168 62		13.55		6 60	
14	500 pounds $CaSO_4$ + 3½ tons lime	167 67	168.14	14.00	13.77	6 60	6.60
15	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	156 35		18.03		3 60	
16	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	155.52	155 93	17.90	17.96	3.10	3 35

Examining this table it is evident that the ammonifying power of the soil organisms was slightly decreased by all the applications of gypsum. Lime alone and lime applied with gypsum in the various amounts increased ammonification, the greatest increase occurring when the gypsum was applied at the rate of 500 pounds per acre.

Gypsum applied alone decreased nitrification, the greatest decrease occurring with the application of 1000 pounds per acre. Lime alone increased the process, but lime with gypsum showed no effect except where the largest amounts of gypsum per acre were used, when a distinct increase occurred.

Azofication was greatly increased with the application of gypsum at the rate of 100 pounds per acre, but the larger amounts showed no effect whatever. Lime increased the process and lime with the various applications of gypsum also showed an increase over the check soil in all cases and over the limed soil in all cases except where the largest amount of gypsum was used.

On the whole, the results obtained at this sampling show no effect from the applications of gypsum alone and when used with lime the increases were generally less than those secured with lime alone. Only in the case of ammonification were the increases from the two materials greater than from the lime alone.

The results secured at the fifth sampling are given in table 6.

TABLE 6

Effect of $CaSO_4$ *on ammonification, nitrification and azofication—Sampling 5*

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	AMMONIFICATION		NITRIFICATION		AZOFICATION	
		NH_3 nitrogen in 100 gm. of air-dry soil	Average	NO_3 nitrogen in 100 gm. of air-dry soil	Average	Nitrogen fixed in 100 gm. of air-dry soil	Average
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None.	134 52		12 1		2.0	
2	None......	134.52	134 52	12 5	12 30	2.0	2 0
3	3½ tons $CaCO_3$	203 55		17 4		4.5	
4	3½ tons $CaCO_3$...	202 60	203 07	17 6	17 50	4 5	4.5
5	100 pounds $CaSO_4$	142.89		9 9		1 5	
6	100 pounds $CaSO_4$	142.07	142.48	9.3	9 60	1.5	1.5
7	500 pounds $CaSO_4$	143 96		6.7		2.0	
8	500 pounds $CaSO_4$.	142 78	143 37	7.7	7.20	2.0	2 0
9	1000 pounds $CaSO_4$.	136 40		10 5		3 5	
10	1000 pounds $CaSO_4$	136 76	136 58	10 3	10.40	3 5	3.5
11	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	209 09		15 5		6 5	
12	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$ ..	209 80	209 44	15.4	15.45	6 5	6.5
13	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$.	207.44		15 7		8 5	
14	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	206 73	207 08	16.3	16.00	8 5	8.5
15	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	172 75		15 0		5 5	
16	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$.	172 28	172.51	14 4	14 70	5.5	5.5

Gypsum applications increased ammonification, the smallest increase occurring when the gypsum was applied at the rate of 1000 pounds per acre.

Lime alone increased the process and when applied with gypsum a still greater effect was noted except where the 1000-pound application of gypsum was made.

Nitrification was decreased with all the applications of gypsum alone, but when the gypsum was applied with lime, increases were secured which were smaller, however, than that brought about by the lime alone.

Azofication was not affected by the smaller applications of gypsum, but the largest amount increased the process slightly. When used with lime, however, distinct increases were obtained, all of which were greater than that given by the use of lime alone.

At this sampling gypsum alone showed an influence only in the ammonification process but when used with lime distinct increases were shown in azofication also. The nitrification process was affected less by the two materials together than by the lime alone.

The results obtained at the sixth sampling appear in table 7.

TABLE 7

Effect of $CaSO_4$ *on ammonification, nitrification and azofication—Sampling 6*

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	AMMONIFICATION		NITRIFICATION		AZOFICATION	
		NH_3 nitrogen in 100 gm. of air-dry soil	Average	NO_3 nitrogen in 100 gm of air-dry soil	Average	Nitrogen fixed in 100 gm of air-dry soil	Average
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None.	136 76		9.4		3 0	
2	None	132 04	134 40	9 4	9 40	3 0	3.0
3	3½ tons $CaCO_3$	211 20		14 6		5.0	
4	3½ tons $CaCO_3$	211 20	211 20	16.0	15 30	5 0	5.0
5	100 pounds $CaSO_4$	120 95		10.4		3.0	
6	100 pounds $CaSO_4$	120 59	120 77	11 4	10.90	3.0	3 0
7	500 pounds $CaSO_4$	134 28		8 0		2.0	
8	500 pounds $CaSO_4$	133 57	133 92	9.1	8.55	2 0	2 0
9	1000 pounds $CaSO_4$	121.54		8 5		1 0	
10	1000 pounds $CaSO_4$	124 49	123 01	8 0	8 25	1 0	1 0
11	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	198 12		14 0		14 5	
12	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	193.28	195 70	14 3	14 15	14 5	14 5
13	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	204 02		12 6		6 5	
14	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	207 32	205 67	12 6	12 60	6.5	6 5
15	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	192 69		13 6		5 0	
16	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	195 29	193 99	13 6	13 60	5 0	5.0

Ammonification was decreased at this sampling by all the applications of gypsum, the greatest decrease occurring with the smallest application. Lime alone showed an increase in ammonification and gypsum with lime also showed a considerable increase over the check soil, but a smaller effect than the lime alone.

Nitrification was also decreased by gypsum alone, except with the 100-pound application, but when lime was used with the gypsum the process was stimulated. The greatest increase in nitrification, however, was secured, as in the case of ammonification, by the application of lime alone.

Azofication was decreased by the larger applications of gypsum, while the application of 100 pounds per acre had no effect. When used with lime distinct increases in azofication were secured, the greatest increase occurring with the smallest amount of gypsum. In every case the two materials showed a greater effect than the lime alone.

At this sampling gypsum alone did not increase the process of ammonification, nitrification and azofication, but in most cases showed slight decreases. When used with lime, however, there were obtained increases in azofication greater than from lime alone. Nitrification and ammonification were not thus affected.

EFFECT OF GYPSUM ON B. RADICICOLA

The results of the tests of the quantities of nitrogen fixed by the pure culture of *B. radicicola* from the nodules of red clover appear in table 8.

TABLE 8

Effect of sterilization of the soil on azofication by B. radicicola from red clover and on nitrification

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	UNSTERILIZED SOIL		STERILIZED SOIL	
		Azofication		Azofication	
		Nitrogen fixed in 100 gm of soil	Average	Nitrogen fixed in 100 gm of soil	Average
		mgm	*mgm*	*mgm*	*mgm.*
1	None	9 25		4 75	
2	None	12 25	10 75	5 25	5 00
3	3½ tons $CaCO_3$	9 75		11 25	
4	3½ tons $CaCO_3$	10 25	10 00	16.75	14 00
5	500 pounds $CaSO_4$	11 25		9 25	
6	500 pounds $CaSO_4$	8 25	9 75	12 25	10 75
7	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	14 25		9 25	
8	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	18 75	16 50	11 75	10 50

The amount of nitrogen fixed by *B. radicicola* in the unsterilized soil was somewhat reduced by the gypsum while the lime brought about practically no effect. The two materials together, however, gave a decided increase. In the sterilized soil both lime and gypsum increased the fixation of nitrogen, the lime giving the greater effect. The influence of the two materials in this case, however, was no greater than the effect of gypsum alone and was smaller than the effect of the lime alone.

The results secured on the amounts of nitrogen fixed by *B. radicicola* from alfalfa, field peas, red clover and soybeans are given in table 9.

TABLE 9

Soil studies on the azofication by B. radicicola, strains of alfalfa, field peas, red clover and soybeans

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	ALFALFA		FIELD PEAS		RED CLOVER		SOYBEANS	
		Nitrogen fixed in 100 gm. of soil	Average	Nitrogen fixed in 100 gm. of soil	Average	Nitrogen fixed in 100 gm. of soil	Average	Nitrogen fixed in 100 gm. of soil	Average
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None	1 15		4.25		0 25		6.75	
2	None	1 15	1 15	4 25	4 25	0.25	0.25	6.75	6.75
3	3½ tons $CaCO_3$	4 25		3 75		2.75		4.75	
4	3½ tons $CaCO_3$	4 25	4.25	3 75	3 75	2.25	2 50	4.75	4.75
5	100 pounds $CaSO_4$	4 25		3.75		1 75		3.75	
6	100 pounds $CaSO_4$	4 25	4 25	3 75	3 75	1.75	1.75	3.75	3 75
7	500 pounds $CaSO_4$	4 25		9 25		1 75		7 75	
8	500 pounds $CaSO_4$	4 25	4 25	11 25	10 75	1 75	1.75	7.75	7.75
9	1000 pounds $CaSO_4$	6 75		8 75		4 25		6 25	
10	1000 pounds $CaSO_4$	6 75	6 75	8 75	8 75	4 25	4 25	6 25	6.25
11	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	6 25		6 75		2 25		7.75	
12	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	5 75	6 00	6 75	6 75	2 75	2 50	7 75	7.75
13	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	4 25		6 25		4 75		8 25	
14	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	4.25	4 25	6 25	6 25	4 75	4 75	8.25	8.25
15	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	4 25		6 25		3 25		6 25	
16	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	4 25	4 25	6.25	6 25	3 25	3.25	6.25	6.25

Observing the table, it is found that the amount of nitrogen fixed by the organisms from alfalfa was increased by the application of gypsum to the soil, the 100-pound and 500-pound applications showing the same effect while the 1000-pound amount gave a greater effect. The lime showed about the same influence as the smaller amounts of gypsum and the two materials together no effect on the single application except with the 100-pound application when a slight gain was noted. In the case of the organism from the field peas the lime and smallest amount of gypsum had no effect, but the larger amounts of gypsum showed a distinct increase. The two materials together had less effect than the gypsum alone except in the case of smallest amount.

The organism from red clover produced an increase in nitrogen-fixing power by both lime and gypsum, the largest amount showing the greatest effect. With the two materials together, slightly greater effects were noted except in one case.

The soybean organism was reduced in fixing power by the lime and 100 pounds of gypsum, but the larger amounts of gypsum had little effect. The two materials together had little effect over the gypsum alone except with the smallest amount when a gain was noted.

It is evident from these results in general that the amount of nitrogen fixed by *B. radicicola* was increased when the larger quantities of gypsum were applied; the smaller amounts frequently showing a decrease. An application of lime with gypsum did not seem to have any pronounced effect on the nitrogen fixed and indeed, in several cases, lime with large amounts of gypsum actually decreased the amount of nitrogen fixed when compared with the effects of large amounts of gypsum applied alone.

The results secured on the nitrogen fixed by *B. radicicola* in solution tests with various amounts of gypsum applied, appear in table 10.

TABLE 10

Solution studies on the azofication by B. radicicola, strains of alfalfa, field peas, red clover and soybeans

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	ALFALFA		CANADA FIELD PEAS		RED CLOVER		SOYBEANS	
		Nitrogen fixed per 100 cc. of solution	Average	Nitrogen fixed per 100 cc. of solution	Average	Nitrogen fixed per 100 cc. of solution	Average	Nitrogen fixed per 100 cc. of solution	Average
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None	2.5		2.7		2.5		1.8	
2	None	2 6	2.56	2.0	2.35	2.5	2.50	2.0	1.90
3	2 tons $CaCO_3$	2.8		1.7		2.3		1.8	
4	2 tons $CaCO_3$	2.9	2.85	1 9	1.80	2.3	2.30	1.7	1.75
5	100 pounds $CaSO_4$	2 2		2.2		1.8		2.0	
6	100 pounds $CaSO_4$	2.2	2.20	2.1	2.15	1.4	1.60	2.9	2.45
7	500 pounds $CaSO_4$	2 6		2.4		2.0		2.9	
8	500 pounds $CaSO_4$	2 5	2.55	1.8	2.10	2.0	2.00	2.3	2.60
9	1000 pounds $CaSO_4$	3 0		2.0		2.5		2.9	
10	1000 pounds $CaSO_4$	2.8	2.90	4.4	3.20	2.4	2 45	3 0	2.95
11	100 pounds $CaSO_4$ + 2 tons $CaCO_3$	2.7		2.5		1.3		2.2	
12	100 pounds $CaSO_4$ + 2 tons $CaCO_3$	3 0	2.85	2.4	2.45	1.3	1.30	2.5	2.35
13	500 pounds $CaSO_4$ + 2 tons $CaCO_3$	2 4		2.5		2 9		2.6	
14	500 pounds $CaSO_4$ + 2 tons $CaCO_3$	2 6	2.50	2.4	2.45	1.8	2.85	2.5	2.55
15	1000 pounds $CaSO_4$ + 2 tons $CaCO_3$	2.8		2.5		1.6		2.1	
16	1000 pounds $CaSO_4$ + 2 tons $CaCO_3$	3 0	2 90	2 3	2 40	1 8	1.70	1.9	2 00

Examining this table it is found that there was no marked increase or decrease in the amounts of nitrogen fixed by any of the cultures either with the application of gypsum alone or with the addition of gypsum and lime together. In a few cases slight increases in the amounts of nitrogen fixed were obtained where the largest amounts of gypsum were applied, but the influence in these cases was not great.

The amounts of nitrogen fixed by *B. radicicola* were much greater in the soil than in the solution, which fact is very likely due to the differences in the soil and the solution as a medium for the growth of these organisms. The liquid medium is hardly as satisfactory for the study of the activities of soil microörganisms, since aeration conditions are generally unsatisfactory for their best activities.

CROP RESULTS

The results of the crop tests of clover and wheat appear in table 11.

TABLE 11

Crop yields—dry weight

NUMBER	POUNDS OF CaSO$_4$ AND TONS OF CaCO$_3$ PER ACRE	WHEAT		CLOVER	
		Dry weight per pot	Average	Dry weight per pot	Average
		gm	*gm.*	*gm.*	*gm.*
1	None	60 00		80 00	
2	None	70 00	65.00	70.00	75.00
3	3½ tons CaCO$_3$	94 50		120 00	
4	3½ tons CaCO$_3$	116.50	105 50	123.00	121.50
5	100 pounds CaSO$_4$	64.00		70 00	
6	100 pounds CaSO$_4$	67.00	65.50	72.00	71.00
7	500 pounds CaSO$_4$	68.00		73 00	
8	500 pounds CaSO$_4$	69 00	68 50	78 00	75.50
9	1000 pounds CaSO$_4$	69.00		134.00	
10	1000 pounds CaSO$_4$	64 00	66 50	131.00	132.50
11	100 pounds CaSO$_4$ + 3½ tons CaCO$_3$	86 00		115 00	
12	100 pounds CaSO$_4$ + 3½ tons CaCO$_3$	89 00	87.50	111 00	113.00
13	500 pounds CaSO$_4$ + 3½ tons CaCO$_3$	70 50		120 00	
14	500 pounds CaSO$_4$ + 3½ tons CaCO$_3$	78 00	74 25	127.00	123 50
15	1000 pounds CaSO$_4$ + 3½ tons CaCO$_3$	80 00		125 00	
16	1000 pounds CaSO$_4$ + 3½ tons CaCO$_3$	78 00	79.00	122 00	123 50

Observing the results given in this table it is evident that the application of gypsum alone did not show any effect on the yield of wheat, but the largest application of gypsum greatly increased the yield of red clover. Lime alone increased the yield of the wheat crop considerably, but when applied with gypsum the increase was much smaller; the effect was greater, however, than where gypsum was used alone. Lime alone and lime with gypsum increased the yields of the red clover crop, but in no case was the increase quite as great as when gypsum was applied alone at the rate of 1000 pounds per acre

Examining the crop yields obtained in tables 13, 14, 15 and 16, it is still more evident that the gypsum applications did not increase to any marked extent the yields of alfalfa, field peas, red clover and soybeans, when these crops were inoculated with the proper cultures of *B. radicicola.*

Taking the crop results as a whole, it is evident that applications of gypsum had very little effect, if any, on wheat or leguminous crops grown on Miami silt loam soil.

THE AVAILABILITY OF PLANT-FOODS

The results obtained on the acidity of the soil and on the production of water-soluble potassium are given in table 12.

TABLE 12

Acidity and soluble potassium

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	ACIDITY		WATER-SOLUBLE POTASSIUM			
		$CaCO_3$ required to correct acidity of an acre	Average	Per cent	Average per cent	Amount per acre	Average amount per acre
		lbs.	*lbs.*			*lbs.*	*lbs.*
1	None	600		0.0037		74	
2	None	675	637	0 0037	0 0037	74	74
3	3½ tons $CaCO_3$	None		0 0075		150	
4	3½ tons $CaCO_3$	None		0 0077	0 0076	154	152
5	100 pounds $CaSO_4$	1625		0 0036		72	
6	100 pounds $CaSO_4$	1625	1625	0 0036	0.0036	72	72
7	500 pounds $CaSO_4$	1875		0.0020		40	
8	500 pounds $CaSO_4$	1850	1862	0 0020	0 0020	40	40
9	1000 pounds $CaSO_4$	1900		0 0048		96	
10	1000 pounds $CaSO_4$	1900	1900	0.0047	0 0047	94	95
11	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	125		0 0099		198	
12	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	100	112	0 0099	0 0099	198	198
13	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	50		0 0098		196	
14	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	200	125	0 0095	0 0096	190	193
15	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	25		0 0093		186	
16	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$		125	0 0090	0 0091	180	183

Observing these results it is found that the acidity of the soil was increased by the application of gypsum, the larger amount giving the greatest increase. Beyond 500 pounds per acre, however, there seemed to be little additional influence on the acidity. The acidity in the untreated soil was much less than when the experiment was begun, probably because of the alkalinity of the tap water used on the soil.

The production of water-soluble potassium in the soil was apparently not affected by the use of smaller amounts of gypsum, but there was a slight increase when 1000 pounds were used. With the 500-pound application there was a slight decrease but the difference was not great. The application of lime alone and lime with gypsum increased considerably the production of soluble potassium, the greatest increase occurring when the smallest amount of gypsum was used, but the difference here was not great. Gypsum and lime together seemed to exert a greater effect than lime alone.

The effects of gypsum on the production of soluble potassium, on the total nitrogen content of the soil and on the total potassium and total nitrogen in alfalfa are shown in table 13.

TABLE 13

Effects of gypsum on the solubility of potassium and on the total nitrogen content of the inoculated soil and crop of alfalfa

NUMBER	GYPSUM ($CaSO_4$) PER ACRE	LIMESTONE ($CaCO_3$) PER ACRE	CROP						SOIL					
			Crop yields		Total nitrogen		Total potassium		Total nitrogen		Water-soluble potassium			
			Per pot	Average	Per cent nitrogen	Average per cent	Per cent potassium	Average per cent	Nitrogen per 100 gm. of soil	Average	Per cent potassium	Average per cent	Pounds per acre	Average pounds per acre
	lbs	*tons*	*gm*	*gm*					*mgm.*	*mgm*				
1	Nil	Nil	3 25		3 08		1 41		93		0 0029		57 80	
2	Nil	Nil			3 30		1 33		93		0 0016		32.16	
3	Nil	Nil	3 30		3 60		1.44		93		0 0022		45 02	
4	Nil	Nil		3 27	3 60	3.39	1 22	1 35	93	93	0 0022	0 0022	45.02	45.00
5	Nil	3½	3.30		3 10		1 30		87		0 0029		57 80	
6	Nil	3½			3 20		1 27		87		0 0016		32.16	
7	Nil	3½	3 00		2 98		1 06		89		0 0016		32.16	
8	Nil	3½		3 15	3 20	3.12	1 09	1 18	89	88	0 0012	0 0018	25.72	36.96
9	100	Nil	3 75		3 48		1 42		93		0 0029		57.80	
10	100	Nil			3 60		1 49		93		0 0022		45.02	
11	100	Nil	2 50		3 60		1 39		93		0 0032		64.32	
12	100	Nil		3 12	3 60	3 57	1 36	1 41	93	93	0 0032	0 0029	64 32	57.86
13	500	Nil	2 50		3 76		1 56		98		0 0016		32 16	
14	500	Nil			3 76		1 41		90		0 0029		57.80	
15	500	Nil	3 25		3 62		1 44		93		0 0029		57.80	
16	500	Nil		2 87	3 62	3 69	1 52	1 48	94	94	0.0025	0 0025	51.45	49.80
17	1000	Nil	3 75		3 54		1 43		93		0 0012		25 72	
18	1000	Nil			4 06		1 06		93		0 0019		38.59	
19	1000	Nil	4 00		3		1 22		92		0 0045		90 04	
20	1000	Nil		3 50	3 66	3 73	0 99	1 17	106	96	0 0054	0 0032	109 34	65.92
21	100	3½	5 40		3 64		0 83		92		0.0041		83 61	
22	100	3½			3 64		0 30		95		0 0041		83 61	
23	100	3½	4 25		3 16		0 67		93		0 0016		32.16	
24	100	3½		4 70	3 50	3 48	0 73	0.76	92	93	0 0016	0 0028	32.16	57.88
25	500	3½	3.25		3 60		0 98		93		0 0016		32.16	
26	500	3½			3.50		0 94		93		0.0019		38.59	
27	500	3½	4 50		3 24		0 70		116		0 0029		57.80	
28	500	3½		3 75	3 10	3 36	0 73	0 84	116	104	0 0025	0.0022	51.45	45.00
29	1000	3½	5.50		4 24		0 95		95		0 0019		38.59	
30	1000	3½			4 14		0.95		94		0 0012		25.72	
31	1000	3½	3.00		3.72		0.90		93		0 0029		57.80	
32	1000	3½		4.25	3.76	3.96	0 90	0.87		94	0.0029	0 0022	57.80	44.98

Examining this table it is quite evident that there was little effect on the crop yields; slight increases were noted when gypsum and lime were applied together. The total nitrogen and total potassium content of the crop was not affected by the application of gypsum.

The total nitrogen content of the soil was not affected by the gypsum except in one case when lime and gypsum at the rate of 1000 pounds per acre were applied together and this result is not conclusive.

The water-soluble potassium in the soil was increased by the application of gypsum and the greatest increase occurred when the gypsum was added at the rate of 1000 pounds per acre. The application of lime alone lowered slightly the amount of soluble potassium in the soil and its use with gypsum in most cases also reduced the action of gypsum alone upon the production of soluble potassium.

The results secured in a similar test with Canada field peas instead of alfalfa appear in table 14.

Observing the results in this table, it is found that the application of gypsum did not increase the crop yield except in one case and, in fact, in most instances a depression was noted.

The total nitrogen and total potassium in the crop was little influenced by the gypsum either alone or with lime, and in the case of the 500 and 1000-pound applications of gypsum alone decreases were noted. Lime alone did not increase the nitrogen content of the crop, but a slight increase in potassium occurred, which disappeared, however, when gypsum was added with the lime.

The application of gypsum had little or no effect on the nitrogen content of the soil, only in one instance showing any appreciable influence.

The water-soluble potassium in the soil was increased by gypsum alone, the greatest increase occurring with the largest application. Lime alone decreased the soluble potassium and when it was applied with gypsum there was an increase which was greater than that of gypsum alone, except where the largest amount of gypsum was used.

The results obtained on the tests with red clover are given in table 15. It may be seen that the crop yield was increased by the 100-pound application of gypsum but when lime was used with gypsum, decreases occurred. The nitrogen content of the crop was not affected by either the lime or the gypsum.

The potassium content of the crop was increased enormously by the gypsum in all amounts. Lime alone had no effect and the two materials together showed no increase over the effect of gypsum alone.

The application of gypsum had practically no effect on the nitrogen content of the soil and lime had only a slight effect. The production of water-soluble potassium in the soil was decreased by the application of gypsum, except where the largest amount was applied, when there was no effect. Lime alone decreased the soluble potassium and when used with gypsum there was no effect except with the 100-pound application, when a slight increase was noted.

The results secured with soybeans appear in table 16. Examining this table, it is found that gypsum applied in amounts larger than 100 pounds per acre decreased the crop yield. When lime was used a slight increase was noted and lime with 100 pounds of gypsum gave a still greater gain. With 1000 pounds of gypsum plus lime, however, a decrease over the effect of lime alone was noted.

TABLE 14

Effects of gypsum on the solubility of potassium and on the total nitrogen content of the inoculated soil and crop of Canada field peas

NUMBER	GYPSUM ($CaSO_4$) PER ACRE	LIMESTONE ($CaCO_3$) PER ACRE	CROP						SOIL					
			Crop yields		Total nitrogen		Total potassium		Total nitrogen		Water-soluble potassium			
			Per pot	Average	Per cent nitrogen	Average per cent	Per cent potassium	Average per cent	Nitrogen per 100 gm. of soil	Average	Per cent potassium	Average per cent	Pounds per acre	Average pounds per acre
	lbs.	*tons*	*gm.*	*gm.*					*mgm.*	*mgm.*				
1	Nil	Nil	10.35		3.16		1.70		92		0.0019		38.59	
2	Nil	Nil			3.16		1 54		92		0.0019		38.59	
3	Nil	Nil	8.70		2.94		1.35		92		0.0012		25.72	
4	Nil	Nil		9 52	2.96	3.05	1.41	1 50	92	92	0 0014	0 0016	28.70	32.90
5	Nil	3½	7 00		3 02		1.96		96		0 0014		28.70	
6	Nil	3½			3.02		1.83		92		0.0016		33.44	
7	Nil	3½	6 30		3 04		1.62		88		0 0012		25.72	
8	Nil	3½		6.65	3 02	3.02	1.60	1.71	88	91	0.0012	0.0013	25.72	28.40
9	100	Nil	8 25		3.04		1.44		100		0 0019		38.59	
10	100	Nil			3 06		1.31		95		0.0025		51.45	
11	100	Nil	9.75		3 00		1 54		89		0.0019		38.59	
12	100	Nil		9.00	2 98	3.02	1 55	1.46	86	92	0.0019	0.0020	38.59	41.78
13	500	Nil	11 90		2.84		1 36		98		0 0019		35.36	
14	500	Nil			2.80		1.35				0.0019		38.59	
15	500	Nil	10.50		2 94		1.06		97		0.0019		38.59	
16	500	Nil		11 20	2.84	2.85	1.06	1.21	97	97	0.0019	0 0019	38 59	37.78
17	1000	Nil	9.60		3.20		0.96		87		0.0025		51.44	
18	1000	Nil					1.12		87		0.0024		48.24	
19	1000	Nil	7.15		2 88		1.46		87		0 0027		54 67	
20	1000	Nil		8.37	2.88	2.99	1.44	1.24	87	87	0 0027	0.0026	54 67	52.25
21	100	3½	5 65				1.86		92		0 0035		70.75	
22	100	3½					1.86		93		0.0036		73 96	
23	100	3½	9.00		2.94		1.46		88		0 0018		37 31	
24	100	3½		7.32	2.94	2.94	1.35	1.63	89	90	0 0018	0.0027	37.31	54.83
25	500	3½	7.25		3.24		1.52		88		0.0041		83.61	
26	500	3½			3.24		1.59		92		0.0038		77.18	
27	500	3½	6.65		3.28		1.60		92		0.0028		57.88	
28	500	3½		6.95	3.28	3.26	1.54	1.56	89	90	0.0028	0.0034	57.88	69 14
29	1000	3½	9.85		2.90		1.55		89		0 0016		32.16	
30	1000	3½			2.90		1.46		89		0.0016		32.16	
31	1000	3½	7.65		2.76		1.57		85		0 0016		32.16	
32	1000	3½		8.75	2.72	2.82	1.39	1.49	86	87	0.0016	0.0016	32.16	32.16

The nitrogen content of the crop was enhanced by the gypsum when used with lime, however there was little increase over the effect of the lime alone. The total potassium in the crop was not affected by the gypsum but when lime was used with the gypsum a decrease occurred.

Gypsum alone had little effect on the nitrogen content of the soil, a slight increase occurring only in the case of the 1000-pound application. Used

TABLE 15

Effects of gypsum on the solubility of potassium and on the total nitrogen content of the inoculated soil and crop of red clover

NUMBER	GYPSUM (CaSO$_4$) PER ACRE	LIMESTONE (CaCO$_3$) PER ACRE	CROP						SOIL					
			Crop yields		Total nitrogen		Total potassium		Total nitrogen		Water-soluble potassium			
			Per pot	Average	Per cent nitrogen	Average per cent	Per cent potassium	Average per cent	Nitrogen per 100 gm. of soil	Average	Per cent potassium	Average per cent	Pounds per acre	Average pounds per acre
	lbs	*tons*	*gm*	*gm*					*mgm.*	*mgm.*				
1	Nil	Nil	5 00		3 36		0.98		93		0.0035		70 75	
2	Nil	Nil			3 38		0 98		93		0.0022		45 02	
3	Nil	Nil	6 35		3 12		0 98		90		0 0022		45 02	
4	Nil	Nil		5 67	3 12	3 24	1 01	0 99	92	92	0 0035	0 0028	70.75	57.88
5	Nil	3¼	3 95		3 08		0 95		99		0.0016		32.16	
6	Nil	3¼			3 08		0 98		99		0 0022		45.02	
7	Nil	3¼	6 60		3 10		0 99		100		0 0016		32.16	
8	Nil	3¼		5 27	3 36	3 13	1 04	0 99	100	99	0.0028	0 0020	57.88	41.80
9	100	Nil	6 75		3 24		2 58		92		0 0019		39.87	
10	100	Nil			3 08		2 50		92		0.0016		32.16	
11	100	Nil	8.30		3 08		2 17		95		0 0019		38.59	
12	100	Nil		7 52	3 08	3 12	2 17	2 35	92	93	0 0035	0.0022	70.75	45.34
13	500	Nil	6 40		3 48		2 37				0 0025		51.45	
14	500	Nil			3 46		2 60		90		0 0032		64.32	
15	500	Nil	5 50		3 48		2 66		97		0 0025		51.45	
16	500	Nil		5 95	3.48	3 42	2 68	2.58	96	94	0 0025	0 0027	51.45	54.66
17	1000	Nil	4 45		3 46		2 31		99		0 0035		70.75	
18	1000	Nil			3 48		2 44		98		0 0025		51.45	
19	1000	Nil	6 05		3 52		2 41		98		0 0019		57.88	
20	1000	Nil		5 25	3 40	3 46	2 54	2 42	98	98	0 0035	0 0028	70 75	57.88
21	100	3¼	4 25		3 52		2 55		98		0.0035		70.75	
22	100	3¼			3 52		2 60		97		0 0032		64.32	
23	100	3¼	4.60		3 44		2 26		99		0.0025		51.45	
24	100	3¼		4 42	3 44	3.48	2 29	2.40	95	97	0 0032	0.0031	64.32	62.71
25	500	3¼	5.50		3.40		1 92		95		0 0019		38.59	
26	500	3¼			3.40		1 96		94		0.0022		45.02	
27	500	3¼	3.75		3 58		2 24		92		0.0032		64.32	
28	500	3¼		4.62	3.48	3 49	2 44	2 14	92	93	0.0029	0 0025	57.88	51.45
29	1000	3¼	2 80		3 36		2 12		97		0.0029		57.88	
30	1000	3¼			3 50		1 99		96		0 0029		57.88	
31	1000	3¼	4.80		3 36		2.15		99		0.0022		45.02	
32	1000	3¼		3 80	3.36	3.39	2.13	2.10	99	93	0.0022	0.0025	45.02	51.45

with lime no additional increase occurred. The soluble potassium in the soil was increased by the use of gypsum but lime caused a decrease. Lime used with the gypsum decreased the soluble potassium.

TABLE 16

Effects of gypsum on the solubility of potassium and on the total nitrogen content of the soil and crop of soybeans

NUMBER	GYPSUM ($CaSO_4$) PER ACRE	LIMESTONE ($CaCO_3$) PER ACRE	CROP: Crop yields: Per pot	CROP: Crop yields: Average	CROP: Total nitrogen: Per cent nitrogen	CROP: Total nitrogen: Average per cent	CROP: Total potassium: Per cent potassium	CROP: Total potassium: Average per cent	SOIL: Total nitrogen: Nitrogen per 100 gm. of soil.	SOIL: Total nitrogen: Average	SOIL: Water-soluble potassium: Per cent potassium	SOIL: Water-soluble potassium: Average per cent	SOIL: Water-soluble potassium: Pounds per acre	SOIL: Water-soluble potassium: Average pounds per acre
	lbs.	*tons*	*gm.*	*gm*					*mgm*	*mgm*				
1	Nil	Nil	9.25		1 32		1 27		105		0 0035		70.75	
2	Nil	Nil			1 48		1 31		105		0.0032		64.32	
3	Nil	Nil	11 00		1 60		1 28		107		0.0030		60.46	
4	Nil	Nil		10.12	1 60	1 50	1 17	1.26	106	106	0.0029	0 0031	57.80	63.33
5	Nil	3½	11.50		1 54		1 27		107		0.0019		38.59	
6	Nil	3½			1.54		1.21		104		0 0019		38 59	
7	Nil	3½	11 25		2 00		1.43		107		0 0032		64 32	
8	Nil	3½		11 37	2.04	1.78	1 39	1 32	103	105	0 0032	0.0025	64.32	51.45
9	100	Nil	11 25		1 16		1 10		101		0.0035		70.75	
10	100	Nil			1 24		1 18		107		0.0035		70.75	
11	100	Nil	9 25		2.24		1 33		114		0 0035		70.75	
12	100	Nil		10 25	2 32	1.74	1 27	1 22		107	0 0035	0 0035	70.75	70.75
13	500	Nil	7 00		1 18		1 27		107		0 0035		70 75	
14	500	Nil			1 34		1 27		112		0 0038		77.18	
15	500	Nil	10 00		2 96		1 18		112		0 0038		77.18	
16	500	Nil		8 50	2 76	2 06	1 14	1 20	112	111		0 0037		77.37
17	1000	Nil	8 50		3.58		1 28		109					
18	1000	Nil			3.48		1 30		109					
19	1000	Nil	9.25		1.56		1 09		108					
20	1000	Nil		8.87	1.52	2 53	1.18	1.21	107	108				
21	100	3½	11.5				1.17		107					
22	100	3½					1.15		112					
23	100	3½	13.5		1.58		0 96		107					
24	100	31		12 50	1 58	1.58	1.06	1.08	107	108				
25	500	3½	12.0		1 60		1.02		107		0.0016		32.16	
26	500	3½			1 76		0.96		113		0.0016		32.16	
27	500	3½	10.0		1 76		1 12		113		0.0029		57.80	
28	500	3½		11.00	1.80	1 73	1.18	1.07	107	110	0.0029	0.0022	57.80	44.98
29	1000	3½	10 25		2.04		1.28		110		0.0016		32.16	
30	1000	3½			2.02		1.02		110		0.0016		32.16	
31	1000	3½	9.75		1.78		1.12		110		0 0022		45.02	
32	1000	3½		10.00	2.04	1.97	1 18	1.15	109	110	0.0022	0.0019	45.02	38.59

DISCUSSION OF RESULTS

A study of the results of the bacterial tests, as summarized in tables 17, 18 and 19, reveals the fact that gypsum applied in various amounts had little effect on ammonification and nitrification in the particular soil, showing in most cases a slight depression in these processes, but azofication was increased, especially when used with lime.

TABLE 17

Summary of ammonification results

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	AVERAGE AMMONIA NITROGEN IN 100 GM. OF AIR-DRY SOIL					
		Sampling 1	Sampling 2	Sampling 3	Sampling 4	Sampling 5	Sampling 6
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None	130.80	96.60	133.40	126.26	134.54	134.40
2	3½ tons $CaCO_3$	150.35	138.38	162.85	151.80	203.07	211.20
3	100 pounds $CaSO_4$	124.95	89.43	130.85	120.65	142.48	120.77
4	500 pounds $CaSO_4$	139.75	102.35	128.25	124.31	143.37	133.92
5	1000 pounds $CaSO_4$	62.90	113.63	135.45	114.26	136.58	123.01
6	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	71.85	154.28	141.60	129.04	209.44	195.70
7	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	72.70	115.77	148.35	168.14	207.08	205.67
8	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	66.6	135.85	158.30	155.93	172.51	193.99

TABLE 18

Summary of nitrification results

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	AVERAGE NITRATE NITROGEN IN 100 GM. OF AIR-DRY SOIL					
		Sampling 1	Sampling 2	Sampling 3	Sampling 4	Sampling 5	Sampling 6
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None	10.50	13.90	18.20	14.67	12.30	9.40
2	3½ tons $CaCO_3$	16.56	15.70	24.50	21.87	17.50	15.30
3	100 pounds $CaSO_4$	8.90	12.20	20.65	12.27	9.60	10.90
4	500 pounds $CaSO_4$	9.30	13.30	14.40	12.37	7.20	8.55
5	1000 pounds $CaSO_4$	10.44	16.40	15.30	9.05	10.40	8.25
6	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	17.30	17.70	17.00	12.97	15.45	14.15
7	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	20.65	17.30	16.20	13.77	16.00	12.60
8	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	20.60	12.25	15.55	17.96	14.70	13.60

The ammonifying power of the soil was depressed, except in a few cases, by the different amounts of gypsum alone, the particular amount exerting the greatest depression varying at the different samplings. The results are extremely irregular and probably definite conclusions should not be drawn along this line. The application of lime alone greatly stimulated this process but the use of gypsum with lime in general gave a smaller effect than the lime alone. The two materials, however, brought about a distinct increase over the ammonification in the untreated soil. Whatever the cause of the injurious effect of gypsum on ammonification it is evidently partly overcome by the presence of the lime. This would seem to indicate that the injury was due to the acidity developed.

The nitrifying power of the soil also was depressed by the gypsum in practically all cases, and in general the larger amounts brought about the greater decreases. Lime alone stimulated the process of nitrification considerably and the application of gypsum and lime together also gave an increase over the nitrification in the check soil, but after the second sampling the stimulation was never as marked as it was when lime was used alone. At the first two samplings there were indications of slight increases from the two materials together over lime alone, but the results are not very definite. Again, the lime seems to overcome the injurious effect of gypsum, in part at least, but even with lime present the gypsum does not seem to exert any beneficial effect.

TABLE 19

Summary of azofication results

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	AVERAGE NITROGEN FIXED IN 100 GM. OF AIR-DRY SOIL					
		Sampling 1	Sampling 2	Sampling 3	Sampling 4	Sampling 5	Sampling 6
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None	1 50	1 20	2 75	2 90	2.00	3 00
2	3½ tons $CaCO_3$	4 50	1.70	2.25	5.60	4.50	5 00
3	100 pounds $CaSO_4$	6 25	4.50	4 75	9.60	1.50	3.00
4	500 pounds $CaSO_4$	4 00	4 80	1.75	2 60	2 00	2.00
5	1000 pounds $CaSO_4$	4.12	6 30	2 25	2 65	3.50	1.00
6	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	10 75	8.30	3 50	6.35	6.50	14.00
7	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	10 55	4 30	3 37	6 60	8 50	6.50
8	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	12 25	2 80	9 75	3.35	5.50	5.00

With few exceptions, the azofying power of the soil was increased by the application of the smallest amount of gypsum alone at the earlier samplings. The larger amounts of gypsum, however, gave definite increases in azofication only at the first two samplings and at the later dates little effect was noted.

Lime alone produced a stimulation in this process but when gypsum was used with lime the increases were much more pronounced. In the case of this process, the gypsum seems to have no depressing effect as on ammonification and nitrification, and hence it would seem that there can be little increase in acidity inasmuch as azofication is sensitive to acidity. Evidently sulfate benefits this process while it has a different effect on the other processes. It may be that the food requirements of the particular bacteria are very different.

The amounts of nitrogen fixed by *B. radicicola* were much greater in the soil than in the solution. Gypsum generally increased the process, the greatest increase occurring with the largest application of gypsum. The fact that a greater amount of nitrogen was fixed in the soil than in the solution is very probably due to the difference between the soil and the solution as a medium for the best bacterial activities.

The crop yields are so extremely variable that conclusions are difficult. In general, however, it seems that gypsum alone had little effect either on wheat or on the various legumes. In a few cases increases were noted and in others slight decreases were evidenced, but in general the differences secured were not very definite. Lime and gypsum together likewise exerted little effect on the crops and in general gypsum used with lime did not increase the yield of legumes over lime alone.

The acidity of the soil was increased by the applications of gypsum alone, the larger amounts producing the greatest increase. When applied with lime, the gypsum brought almost a slight acid condition in the soil, whereas the limed soil gave a neutral reaction. It would seem evident from these results that gypsum does make the soil acid and when used lime should be applied in sufficient amounts so that no injurious effect can occur.

TABLE 20

Production of water-soluble potassium in the soil

NUMBER	POUNDS OF $CaSO_4$ AND TONS OF $CaCO_3$ PER ACRE	AVERAGE WATER-SOLUBLE POTASSIUM PER ACRE IN FALLOW SOIL	AVERAGE WATER-SOLUBLE POTASSIUM PER ACRE IN INOCULATED SOILS GROWING CROPS OF			
			Alfalfa	Canada field peas	Red clover	Soybeans
		lbs.	*lbs*	*lbs.*	*lbs.*	*lbs.*
1	None	74	45	32 90	57 88	63.33
2	3½ tons $CaCO_3$	152	36 96	28 40	41 80	51.45
3	100 pounds $CaSO_4$	72	57 86	41 78	45 34	70.75
4	500 pounds $CaSO_4$	40	49 80	37 78	54 66	71.37
5	1000 pounds $CaSO_4$	95	65 92	52 25	57 88	
6	100 pounds $CaSO_4$ + 3½ tons $CaCO_3$	198	57 88	54 83	62.71	
7	500 pounds $CaSO_4$ + 3½ tons $CaCO_3$	193	45 00	69 14	51 45	44.98
8	1000 pounds $CaSO_4$ + 3½ tons $CaCO_3$	183	44 98	32 16	51.45	38.59

The production of water-soluble potassium in the fallow soil was apparently not affected by the use of smaller amounts of gypsum as shown in table 20, but there was a slight increase when the largest application of gypsum was used. The application of lime alone and lime with gypsum increased considerably the production of soluble potassium in the fallow soil. Gypsum and lime together seemed to exert a greater effect than lime alone.

The production of soluble potassium in the soil where the leguminous crops were grown was increased in general by the applications of gypsum alone, the largest increase occurring with the largest addition of gypsum. The application of lime alone, in all cases decreased the production of soluble potassium in the soils where legumes were grown but the use of gypsum with lime sometimes brought about an increase. Again the results are somewhat variable but in general the smaller amounts of gypsum with lime proved beneficial, while the larger quantities showed little effect.

The utilization of soluble potassium in the crops would not explain the difference between these results and those secured on the fallow soil, and hence it would seem that the results are not sufficiently definite to warrant conclusions. The use of gypsum alone, however, apparently brings about distinct increases in the production of water-soluble potassium, and hence the beneficial effect of the material on crops may sometimes be due to this action.

SUMMARY

The results of these experiments lead to the following conclusions:

1. Ammonification was decreased by the application of gypsum alone, the greatest decrease occurring with the largest application of the material. Lime favored ammonification and lime with gypsum showed less effect in general than lime alone.
2. Nitrification was similarly depressed by gypsum alone but the use of gypsum and lime together increased this process.
3. One-hundred-pound applications of gypsum stimulated azofication and the larger amounts also stimulated the process in most cases, but to a less extent than the smaller amount alone increased azofication.
4. The amounts of nitrogen fixed by *B. radicicola* were much greater in the soil than in the solution. Gypsum generally increased the process, the greatest increase occurring with the largest application of gypsum.
5. The 1000-pound application of gypsum increased the yield of red clover; other applications, however, did not have any effect either on the wheat or on the other leguminous crops. Lime alone increased the yield of wheat and clover considerably.
6. The application of gypsum increased the acidity of the soil, the larger amounts giving the greatest increase.
7. The nitrogen and the potassium content of the leguminous crops were not affected by gypsum, except in the case of red clover when an enormous increase in its potassium content was noted.
8. The nitrogen content of the soil was not markedly affected by gypsum.
9. A slight increase occurred in the production of soluble potassium in fallow soil by the addition of 1000 pounds of gypsum alone, but the other applications did not show any effect. Lime alone increased the soluble potassium considerably and lime with gypsum gave increases over the lime alone.
10. When leguminous crops were grown the production of soluble potassium was greatly increased by the use of gypsum alone, the greater increase occurring with the larger applications.

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THE WATER-SUPPLYING POWER OF THE SOIL AS RELATED TO THE WILTING OF PLANTS[1]

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ABSTRACT

This paper reports a preliminary study of soil moisture conditions considered from a dynamic point of view, as these are related to the wilting of ordinary plants. These conditions affect the plant directly through the water-supplying power of the soil, by which is meant the capacity of the soil to operate as a system or machine delivering water to the absorbing surfaces of the plant roots. Of course, this water-supplying power of the soil, for any given temperature, depends, in its turn, upon a complex of static conditions, such as the sizes and kinds of the solid particles in the soil, their arrangement (packing) and the water content per unit of soil volume. It is impossible, however, to derive from such of the static soil features as can be measured and expressed in quantitative terms, any single numerical expression for the water-supplying power of a given mass of soil. It has therefore seemed desirable to approach this whole problem from the dynamic side and to attempt to measure the water-supplying power directly, somewhat as the evaporating power of the air is measured by means of atmometers. If this might be accomplished it would make it possible to study the daily or seasonal march of soil water-supplying power in a manner similar to that in which the evaporating power of the air may now be studied.

The method employed in this preliminary study involved the use of small, porous-porcelain cones all having approximately the same area of surface in contact with the soil. At the time of a determination a weighed, dry cone is thrust into the soil and left for a suitable time period, after which it is removed, quickly brushed free from adhering soil, and reweighed. The amount of water absorbed from the soil by the instrument is taken as an approximate measure of the water-supplying power of the soil. This way of approaching the important problem of soil moisture is new, as is also the device employed, but the results thus far secured indicate considerable promise for future developments.

Plants of *Coleus blumei* and of wheat (*Triticum sativum*) were grown in twelve different soils, including a heavy loam, a sand and a very pure humus,

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[1] Botanical contributions from the Johns Hopkins Hopkins University, no. 64.

together with various mixtures of loam and sand, loam and humus, and sand and humus. When the plants were in healthy condition the watering of the cultures was discontinued and wilting was allowed to occur. At or about the critical stage of "permanent wilting" the water-supplying power of the soil about the roots was determined by the new method. It was found that this value was from 0.04 to 0.11 gm. (average, 0.085) for 2 hours and for the porous porcelain cones used. The average was the same for Coleus as for wheat. In the case of Coleus the variations of this value from culture to culture were not at all related to the kind of soil or to its water-holding power; sand, loam and humus all agreed, when the plants were about the critical point, in giving a value of 0.09. In the case of wheat there is some suggestion that the soils of higher water-holding power gave very slightly higher values than did the others; for sand, loam and humus the corresponding values were 0.04, 0.09 and 0.10, respectively. But each test is based on only a single determination, so that this is but a suggestion. In any event, the index of water-supplying power may be regarded as practically the same for all twelve soils. It will probably be found to differ markedly for different kinds of plants (although it seems to be about the same in the cases of Coleus and wheat), for the same kind of plant grown under different moisture conditions, and for plants of the same kind grown under the same moisture conditions but brought to permanent wilting under different moisture conditions. The water-supplying power of all these soils, at the beginning of permanent wilting in these plants, was between 0.1 and 0.5 per cent of the water-supplying power just after a thorough watering of the pots, this latter value being the same for all the soils tested.

INTRODUCTION

The subterranean environmental moisture complex is directly influential upon plants through the water-supplying power of the soil, as has been emphasized by Livingston (6, 7, 12, 14). As far as plant water-relations are concerned, a soil is satisfactory for growth if it can supply water to the root system as rapidly as the latter can absorb it; the rate of external water supply at the absorbing surfaces does not become a limiting condition for growth until this rate falls below the rate of absorption demanded by the internal conditions of the plant. If, however, the soil is not capable of supplying moisture to the root system as rapidly as the latter can take it up, then the soil-moisture complex becomes a limiting condition for growth. As long as such a state of affairs prevails the growth of the plant, and other features of its activity, must be more or less markedly different from what they would be if water were more rapidly furnished to the roots.

The capacity of a given soil mass to deliver water to the root system of a plant rooted in it, might be relatively determined and defined as the maximum possible rate at which that particular soil mass (at the given instant) is capable of delivering water to some standard water-absorbing surface. Relative measurements of the water-supplying powers of soils might be expressed in terms of the number of milligrams of water that could be delivered, in a unit of time, at a square centimeter of absorbing surface within the soil. Of course the actual absorbing surface used must be capable of removing the water as rapidly as it is delivered. It is obvious that the water-supplying power of a given body of soil at any given instant may vary from place to place within the soil (as at different depths, etc.), and that the numerical index of this soil condition must alter in value from time to time for any given region in the soil.

This dynamic point of view with regard to soil moisture is very different from the one heretofore generally taken in soil science and plant physiology. It promises to open the way to a definite advancement of our knowledge of the water relations of plants, in somewhat the same way as did the introduction of a corresponding point of view regarding the moisture conditions of the air (6, 8, 9). The experiments reported in this paper were planned as a working test of one method by which the water-supplying power of the soil may be studied directly, somewhat as the capacity of a machine to do the work of supplying water to the plant roots. A number of other methods have suggested themselves to one of the writers, from time to time during the period since 1904, and two or three of them have received some attention in the literature (6, 13, 14). The ones previously tested have shown themselves to be more suitable for refined laboratory work on artificially prepared masses of soil than they are for the field operations in regard to which adequate measurements of this dynamic soil condition are so greatly needed. The method resorted to in the present experiments is much simpler in its conception and much more satisfactory in use than any other that has thus far been suggested in the literature, and it has considerable promise for field work. This paper has been prepared partly to bring the dynamic point of view more into the prominence which it deserves in these connections, and partly with the hope that other interested workers may test and improve the method here introduced, the present writers being unable to carry the work farther in the immediate future.

As in the case of other conditions that directly affect the plant, the water-supplying power of the soil is dependent upon a complex of secondary conditions, including the static features generally discussed in connection with the subterranean water relations of plants. For any given small region in any given soil, at any given instant of time, the water-supplying power is clearly a function of a considerable number of measurable characters. Some of these static features are capable of definite description, but not all of them (12) can be readily characterized by single numerical indices, and the intrinsic difficulties here encountered are so great that there is no apparent hope for any indirect derivation of the index of the water-supplying power through the employment of secondary indices. If the kinds, sizes and arrangement of the solid soil particles might be represented by a single numerical value and if the soil water content might be similarly stated, it would still be necessary to know just how to combine these two secondary values in order to obtain a true index of the water-supplying power. One of these two soil features, the water content, can be nicely, but somewhat laboriously, determined for any given region in a field soil by the method devised by Pulling (13). The work of obtaining information on this point for hundreds of soil samplings (at different times, for different depths, etc.) is at best not inviting, however. The other important static feature (or group of features) that would need evaluation for an indirect computation of a numerical value for the water-supplying power, is still farther beyond us at present; no satisfactory method for its estimation has been even suggested. The mechanical analysis throws no helpful light on the subject. Determination of the water-holding power or of the water equivalent would require that this static feature should be measured without disturbing the arrangement (packing) of the soil particles, and this is not readily feasible at present. On the whole, the indirect determination of the water-supplying power (the only soil feature that directly influences plants, as far as their water relation is concerned) is quite hopelessly out of the question at present, even if it were desirable to approach the problem in such a round-about manner.

The calculation of a numerical index for an immediately effective environmental condition, from values representing conditions that are not immediately effective upon the plant (conditions that influence the plant only through their determination of the effective condition, which, in turn, influences the plant), is logically undesirable in every case. It is far more satisfactory to measure the intensity and duration of the effective condition directly, whenever this can be done. Thus, the evaporating power of the air may be directly measured by atmometric methods, without dealing with measurements of air temperature, air moisture and air movement, and without attempting the very difficult operation of integrating these three data to give an index of evaporating power. From such considerations as those suggested above it becomes increasingly clear that feasible ways for making direct measurements of the water-supplying power of the soil are among the greatest present desiderata in soil science, plant physiology, ecology, etc. This dynamic condition cannot be evaluated by indirect means and it would be undesirable to evaluate it indirectly even if that were possible and feasible.

POROUS-PORCELAIN SOIL-POINTS FOR THE QUANTITATIVE DETERMINATION OF THE WATER-SUPPLYING POWER OF THE SOIL

The new method for directly obtaining a single numerical index of the dynamic soil feature here emphasized is based on the fact that dry, unglazed, porous porcelain exhibits a remarkable capillary attraction for water, so that this material may be made to present a good water-absorbing surface to the soil. Our method consists, in short, in placing an "artificial root," made of dry porous porcelain, in the soil and determining how much water is absorbed in a given time period. It is desirable that determinations be made as easily and as rapidly as possible, and the soil must not be disturbed more than is absolutely necessary in making a determination. Also, determinations of the water-supplying power of the soil need to be made for various depths as well as for different positions in a horizontal plane.

With these ideas in mind, the "artificial roots" were planned as hollow cylinders of porous porcelain (similar to the material out of which porous-porcelain atmometer cups, etc., are made) having one end open while the other is closed and tapers to a conical point. A portion of the surface nearest to the pointed end is water-proofed, as is also the entire cylindrical surface and a ring of the conical surface adjacent to its junction with the cylindrical part. There is left, for water absorption, a conical surface that may be readily brought into close contact with the soil at any depth. Both the water-proofed and the absorbing surfaces are of such nature that clinging soil particles may be easily removed by brushing, and the porcelain is sufficiently resistant so that there is practically no danger of any of this material being lost. The instrument is first dried and weighed in the dry condition, it is then inserted in the soil at the place to be investigated, and left for a suitable time period, after which it is removed, brushed and reweighed. The increase in weight is a measure of the amount of water absorbed from the soil. The instrument can be used repeatedly, but of course requires to be dried before each application. These "artificial roots" have been termed *soil-points* for the present discussion; a better term may be evolved later if the instrument becomes generally used.

It is seen at once that this method involves the use of a number of soil-points in the same study and that it is desirable that they should be as nearly alike in their water-absorbing power as is possible. This means that the water-absorbing part should be of the same sort of material and that this surface should have the same area and form in all instruments. It is possible that different kinds of material and different sizes and forms of water-absorbing surface may be used interchangeably if suitable coefficients of correction have been obtained by preliminary standardization, but this possibility has not yet been studied.

The soil-points thus far available are 13.5 cm. long, the cylindrical portion being 8 cm. long and 2.5 cm. in outside diameter. The wall is about 3 mm. thick. The conical portion is 5.5 cm. long and tapers from a point to an outside diameter of 2.5 cm. They are water-proofed from the tip for a distance of about 1 cm. and the absorbing portion extends from the margin of this water-proofed area to the lower margin of the water-proofed upper portion, the conical absorbing surface being 2. cm. high, 1.1 cm. in diameter at one end and 2 cm. in diameter at the other. The absorbing surface therefore has an area of proximately of 10 sq. cm.

To insert the points in pots of soil without pebbles or other obstacles it is necessary simply to plunge them in from the free soil surface, after the manner of setting a stake in soft soil. They must be inserted far enough so that all of the absorbing surface shall be in contact with the soil; otherwise this surface may be placed at any desired depth. When the depth of the soil region to be studied is so great that the whole porcelain piece comes to lie below the free soil surface, a special device for placing and recovering the soil-point is necessary, but this exigency was not encountered in these preliminary tests and ways of meeting it need not be discussed here.

If the soil to be studied offers obstacles to the easy penetration of the soil-points it is desirable to make an opening first, of the proper size to receive the instrument. Perhaps a metal replica of the soil-point may be best suited for this. In our work we have made the preliminary opening in the soil by means of one of the soil-points that had been completely water-proofed, so as to have no absorbing surface. For some of the harder soils a cylindrical section was first removed with a cork-borer. Our soils contained no pebbles.

In thrusting the soil-point into the soil the conical portion acts, as it moves downward, to displace the soil laterally and to compress it to some extent. This disturbance can not be avoided in using any method of the sort here considered, but the compression produced should be similar for all tests. For this reason, and for others, it may be ignored for the present. It may be added that this artificial compression of the soil near the surface that is to be tested is not unlike what occurs as a root elongates and forces its tip into new regions of the soil. The compression should act generally to render the readings of the instrument a little higher than would be the case if the soil were not compressed.

When the absorbing surface of the soil-point has reached the desired depth it is left in position for the standard time period, care being taken, of course, not to tilt the instrument laterally and thus break the capillary continuity between the soil and any part of the conical surface. We have found that two hours is satisfactory as a standard time of exposure in cases where the water content of the soil tested does not approach the content that satisfies the water-holding power of the soil. When the soil is very wet a shorter time should be employed.

At the end of the period of exposure the soil-point is removed from the soil and any clinging soil particles are brushed off, after which it is placed in a weighed celluloid cylinder of suitable size, with sliding cap. The final weighing should be accomplished without removing the instrument from its case. After this weighing the porcelain piece is removed from its case and dried at 102°–105°C., for 24 hours or longer. It is then taken from the oven and cooled in a desiccator, being finally returned to its case for transmission to the place where the next exposure is to be made. It is well to store the soil-points generally in a desiccator, although the celluloid cases are practically sealed against water absorption.

EXPERIMENTATION AND RESULTS

In order to be able to test soils of many different natures, we employed a fine glass sand, a somewhat clayey loam and a humus containing very little mineral matter, together with mixtures of sand and loam, sand and humus, and loam and humus. Three mixtures were used for each combination, the two components having the volumetric proportions of 1:3, 1:1 and 3:1. There were thus twelve different soils in the series. The water-holding power of each soil was determined by the Hilgard method (1-cm. column), and it was found that the twelve soils might be arranged so as to form a rather even series on the basis of this soil feature. They were numbered in the order of their values for water-holding power, and their numbers, together with the proportions of the three components in each case, and the corresponding water-holding-power values, are shown in table 1. The water-holding capacity is shown as percentage of the dry volume and also as percentage of the dry weight. It is seen that soil 1 is sand (with a volumetric water-holding power of 40.7 per cent), no. 6 is loam (with a corresponding value of 57.7 per cent) and no. 12 is humus (with a value of 103.1 per cent).

Since the soil feature under consideration is to be dealt with as an indication of the relation between living plants and the moisture conditions of the soil about their roots, the new method here brought forward needs to be tested by comparison with similar measurements obtained by biological tests. If the reasoning of the preceding paragraphs is correct and if our proposed method for approximating the water-supplying power of the soil is satisfactory, as a means of describing a soil as the latter might affect plants, then it should follow that soils giving similar water-supplying powers ought to exert similar influences on the growing plants used as biological indicators. In order to test this proposition it is desirable to choose some critical condition of the plant in its response to soil moisture conditions and to determine the water-supplying powers of various different soils at times when all the plants are in the critical condition. As has been said, the soil-moisture complex of conditions does not directly influence the plant unless the water-supplying power of the soil has a value low enough so that this becomes a limiting condition, for the plant used and for the particular complex of aerial conditions, etc., that is operative in the test; in other words, unless the soil fails to supply moisture to the roots as rapidly as they would absorb it if it were adequately supplied. For any complex of non-soil-moisture conditions and for any set of internal conditions in the plant, an inadequate water-supplying power should become notably influential upon the organism through cessation of growth, progressive decrease in turgor, wilting, and dying of the tissues. The degree of incipient drying (of Livingston and Brown (11)), is not so readily measured quantitatively as are some of the symptoms following the beginning of wilting. The beginning of wilting itself is a critical condition in the plant, but it is very difficult to be sure just when a given individual plant begins to wilt. This is

TABLE 1

Results of experiments on the relation of the water-supplying power of the soil and the wilting of plants

		SOIL NUMBER											
		1	2	3	4	5	6	7	8	9	10	11	12
I. Volumetric proportions of the three soil components	Sand....	Only sand	3	1	1	3	......	...	1	..	1	.. ...	......
	Loam ...		1	1	3	....	Only loam	3	.....	1	.....	1	......
	Humus....			..	.....	1	.....	1	1	1	3	3	Only humus
II. Water-holding power of soil (1-cm. column), *per cent*	Volumetric, on dry volume (A)....................	40 7	43 0	45 8	50 7	55 7	57 7	67.5	71.1	79.5	81.4	86.5	103.1
	Gravimetric, on dry weight (a)....................	28.9	32 8	37 0	43.6	43 9	56 8	70.3	64.4	90.6	91 0	114.0	140.5
III. Water-supplying power of drained saturated soil (10-cm. column) by soil-points. (*Hundredths gram* for 2-hour period, calculated from ½ hour period.) (Average of averages, 1412)	First test	1448	1488	1224	1452	1776	1220	1440	1492	1 84	1468	1840	1196
	Second test...............	1540	1204	1316	1384	1292	1676	1308	1188	1328	1464	1224	1760
	Average (B)...............	1494	1346	1270	1418	1534	1466	1374	1340	1276	1466	1482	1478

			1	2	3	4	5	6	7	8	9	10	11	12
IV. Water content of soil at critical wilting stage, *per cent.* (Critical content-residue)	Volumetric, on dry volume (C)	Coleus	2.3	2.5	6.1	8.9	11.8	13.1	18.5	22.9	26.7	30.2	33.2	48.8
		Wheat..	1.6	2.5	5.0	6.2	7.6	9.3	16.7	17.5	23.2	28.7	32.9	46.2
	In terms of volumetric water-holding power (C/A)	Coleus. ...	0.06	0.06	0.13	0.18	0.21	0.23	0.28	0.32	0.33	0.37	0.38	0.47
		Wheat. .	0.04	0.06	0.11	0.12	0.14	0.16	0.25	0.25	0.29	0.35	0.38	0.45
	Gravimetric on dry weight (c)	Coleus. .	1.4	1.7	4.9	7.3	8.0	10.4	17.6	19.9	28.2	32.1	40.1	70.7
		Wheat.	1.0	1.7	3.7	4.7	5.1	7.4	15.5	13.9	24.7	28.0	39.7	58.1
	In terms of gravimetric water-holding power (c/a)	Coleus.... .	0.05	0.05	0.13	0.17	0.18	0.18	0.25	0.31	0.31	0.35	0.35	0.50
		Wheat.... .	0.04	0.05	0.10	0.11	0.12	0.13	0.22	0.22	0.27	0.31	0.35	0.41
V. Water-supplying power of soil at critical wilting stage, by soil-points. (Critical supplying-power-residue)	*Hundredths gram,* for 2-hour period (D)	Coleus (Average, 8.5,	9	10	6	10	7	9	10	7	. ...	7	9	9
		Wheat (Average, 8.5)	4	6	7	7	8	9	10	9	11	10	11	10
	In terms of supplying power of drained saturated soil (D/B)	Coleus (Average, 0.006)...	0.006	0.007	0.005	0.008	0.005	0.006	0.007	0.005	.....	0.005	0.006	0.006
		Wheat (Average, 0.006)...	0.003	0.004	0.006	0.005	0.005	0.006	0.007	0.007	0.009	0.007	0.007	0.007

especially difficult when different kinds of plants, or differently grown individuals of the same kind, are to be compared. From a group of various kinds of plants that are beginning to suffer from progressive incipient drying it is almost impossible, as yet, to separate those in which wilting has actually begun from those that are not yet actually wilting and those that have already passed the beginning of wilting.

The only critical stage in progressive wilting that has thus far received much attention in the literature is the beginning of what has been called *permanent* wilting (1, 2, 3, 4). Permanent wilting has been defined as that stage of progressive wilting from which recovery fails to occur within a period of 24 hours, if the wilted plants are exposed to a practically water-saturated atmosphere (in darkness) during that period. To determine whether a given plant is permanently wilted, it is thus necessary to subject it to this 24-hour exposure to saturated air. But this procedure throws no light on the question as to whether the plant may not have already passed the *beginning* of permanent wilting. It may be much more seriously affected than it was at the beginning of this stage; indeed, a totally dead plant would fulfil the conditions of the saturated-air test, for it surely would not recover during the test period.

By considerable practice, however, it is possible for an observer to gain such facility of visual judgment as to be able to detect the beginning of permanent wilting with a fair degree of precision in a form with which he is familiar, without resorting to the saturated-air test, except as a check on his visual observation (1, 2, 3, 4, 5, 10, 15). We took advantage of this fact and adopted the beginning of permanent wilting as the most feasible critical condition to employ in the comparative tests. Healthy plants were grown in each of the twelve different soils already described, watering was discontinued when they had attained a good growth, and wilting was allowed to supervene and to progress until the permanent stage had just been attained. When permanent wilting had just begun the soil-point was applied to the soil and a measurement of the water-supplying power was obtained. If our determination of the beginning of permanent wilting were sufficiently precise, and if the soil-point method were satisfactory, all readings obtained from the soil-points should be approximately the same, without any reference to what kind of soil might be involved. Of course it is to be noted that the beginning of permanent wilting for any kind of plant occurs sooner when the organism is rooted in a soil with low water-holding power than when it is rooted in a soil with higher water-holding power, other conditions being alike in both cases, so that such tests as we are dealing with could not all be made on the same day. The plants in sand reached the critical stage of wilting several days before those in humus. The aerial conditions of the greenhouse room in which our cultures stood were nearly the same throughout the period of wilting, and all cultures of a series had been subjected to the same aerial conditions throughout their earlier period of growth. The plants were as nearly alike as possible at the time watering was discontinued. Of course, there were chemical differences between our different soils, but this feature was purposely neglected in this study. There are many reasons for thinking that these chemical differences were practically without influence in determining the relation between the beginning of permanent wilting of the plants and the corresponding soil-point values.

The plants used in these tests were *Coleus blumei* and wheat (*Triticum sativum*). The Coleus plants had been propagated by cuttings and had grown for several weeks in 4-inch pots of the various soils described above. The wheat plants were grown from seed planted directly in the potted soils. Both varieties were well grown and apparently healthy when watering was discontinued. Wilting was allowed to progress until the critical stage corresponding to the beginning of Briggs and Shantz's permanent wilting had been attained, previous experience with similar plants and the saturated-air test having made it possible to detect the advent of this critical stage with considerable precision. The beginning of permanent wilting is more easily and definitely detected by visual observation in the case of Coleus than, in that of wheat and it is probable that the Coleus plants were more nearly alike in their degree of wilting at the time of the soil tests than were the wheat plants; this matter will be referred to in connection with the results.

Twelve different soil-points were used. They had been subjected to several comparative tests in the same mass of soil and had given evidence of being about alike in their water-absorbing powers. As it finally developed, there were some differences in absorbing power, but these may be neglected in the present stage of our knowledge of this new method. These differences were probably no greater than the unavoidable differences between the surface contacts formed with the soil in different tests, or than the inaccuracies involved in determining just when the plants were at the critical stage of wilting that was desired.

At the time the tests were made, soil samples from the various pots were obtained, by means of a cork-borer—in the manner described by Shive and Livingston (15)—and the water content of each soil was determined, both on the volumetric and on the gravimetric basis. These water-content values (expressed as percentages of the dry volume or dry weight of the soil) correspond to the so-called "wilting coefficients" of Briggs and Shantz (12). This index may be called the *permanent-wilting content-residue, or critical content-residue*, of soil moisture; the values do *not* represent "non-available" water, since plants undoubtedly often continue to absorb moisture from the soil at a considerable rate long after permanent wilting has set in and even after they are dead. In table 1, section IV, are shown the critical content-residue values for the 24 different pots—12 different soils for each kind of plant. Besides showing these content-residues as represented on the usual, volumetric and gravimetric basis (C and c), table 1 (section IV) also presents each value in terms of the corresponding water-holding power of the soil (C/A and c/a). This fractional or ratio index may be called the *critical content-residue fraction*, of soil moisture.

The values obtained by means of soil-points applied at the roots of the permanently wilted cultures, are shown in table 1, section V (D), as indexes of the water-supplying powers of the several soils at the time when permanent wilting had begun. These values are stated in terms of *hundredths of a gram* of water absorbed by each soil-point during an exposure period of 2 hours, but they are to be regarded merely as relative values. This index may be called the *critical supplying-power-residue.*

Finally, the values for the water-supplying power were obtained for each of the twelve soils when it had just been saturated and drained, in a 4-inch pot (10-cm.-column of soil). The pots were plunged in water for 2 hours, and then drained in a moist chamber for an hour, after which the soil-points were applied in the usual manner, excepting that the exposure period was shortened in this case to 30 minutes. Since the entrance of water into the porous porcelain of the soil-point acts directly to decrease the water-absorbing power of the material, and since there is comparatively little porous porcelain available in the soil-point here employed, it is clear that this instrument ought not to be used with a 2-hour exposure period when the water-supplying power of the soil is very high. This matter will require future investigation, and will need to be borne in mind as the soil-point method is improved; in the present case the readings were obtained for half-hour periods and each half-hour value was multiplied by 4, to give a derived 2-hour value. These latter are presented in table 1, section III. Two separate tests are represented in each case and the average value for each soil is shown in the lower line of this section of the table (B). This index may be termed the *greater* water-supplying power of the soil. Its value may be considered as approaching the *maximum* water-supplying power in each case, although the magnitude of the maximum for any soil mass is probably influenced by the surroundings, outside of the mass itself (for example, the kind of pot used, etc.). At any rate, whatever details further study may bring out, each of these *greater* supplying-power values may be taken to represent the condition of a pot of the corresponding soil when it has very recently been thoroughly watered, but after subterranean run-off has ceased.

Corresponding to what we have called the critical content-residue fraction, we have derived a *critical supplying-power-residue fraction* for each soil and plant, this being the ratio of the critical supplying-power-residue to the corresponding greater supplying-power, and the values of this index are shown in table 1, section V (D/B).

It may be remarked that the special terms just defined have been selected for their descriptive value rather than for simple brevity. After workers in this field have become generally familiar with these concepts the terms may be shortened and allowed to become less clearly descriptive. It is not our aim to promulgate any special terminology, although we venture to hope that

Greek words may not be introduced to replace our English ones; it is desirable that scientific terminology should be no more pedantic than is really necessary for precision. Perhaps the final outcome may be that letters may be employed in place of our somewhat cumbersome and obviously built-up expressions, just as has long been true in certain branches of physics.

From the data given in table 1 it is at once evident that the critical content-residue (C or c) and the critical content-residue fraction (C/A or c/a) both agree with the water-holding power (A or a) in that they show a progressive increase in magnitude from a minimum index value for the sand to a maximum for the humus soil. As was to be expected, the higher the water-holding power of the soil, the higher is the critical content-residue. The volumetric water-holding power varies from 40.7 to 103.1 (or from unity to 2.5), and the corresponding critical content-residue varies from 2.3 to 48.8 (or from unity to 21.2) for Coleus and from 1.6 to 46.2 (or from unity to 28.9) for wheat. The critical content-residue (representing the soil moisture content at the time of the inception of the permanent wilting stage) is thus seen to increase more rapidly throughout the series of soils than does the water-holding power. The former is 21 or 29 times as large, while the latter is only 2.5 times as large, for the humus as for the sand. Roughly speaking, the critical content-residue increases in magnitude about ten times as rapidly, from sand to humus, as does the water-holding power. The volumetric content-residue fraction varies from 0.06 to 0.47 (from unity to 7.8) for Coleus and from 0.04 to 0.45 (from unity to 11.3) for wheat. Therefore, the value of this ratio increases more rapidly than does the index of water-holding power, but not as rapidly as does the index of the critical content-residue. Both the content-residue and the content-residue fraction are apparently determined by conditions that differ from soil to soil, so that neither one of them can be of any direct use in predicting the onset of permanent wilting. Nor can the water-holding power be used for this purpose, as has long been known, unless its index is somehow combined with the index of the critical content-residue, and these data show at least that the quotient of the latter divided by the former (the critical content-residue fraction) does not represent a way to accomplish this combination.

It is to be remembered that all these soils are to be considered as practically alike at the beginning of permanent wilting for either plant; by the biological test the entire twelve were alike in their moisture conditions as far as these affect wilting. It follows that any index or criterion of the soil-moisture complex that might be used for predicting the critical point of wilting ought therefore to show like values for all soils when the plants are all in the critical condition. Turning again to table 1 (section V, D), it is seen that this is exactly what the critical supplying-power-residue does show. This is best demonstrated for Coleus, for which the value of this index is exactly the same (9) for sand, loam and humus. Its variations, while apparently considerable (the minimum is 6 and the maximum is 10 in this case), bear no suggestion of a relation to the progressively increasing series of values that have just been considered. In this regard the data for wheat are a little less definite in their indications, but it is more difficult to detect the beginning of permanent wilting in this plant than in Coleus, as has been said. From no. 6 to no. 12 in the soil series, the wheat data for the critical supplying-power-residue give the same indication as has just been pointed out for the entire series in the case of the other plant, but for the lower soil numbers this index value tends to be lower with lower water-holding power. As will be shown presently, however, the total variation (between 4 and 11) is really negligible in the present connection. Also, it seems probable that the wheat plants of soil 1 had progressed somewhat farther in the drying-out process than would be represented by the beginning of permanent wilting. It may be more nearly correct to consider the critical supplying-power-residue for soil 1 as 6 rather than 4, in which case the total variation would become less. (Making a similar arbitrary alteration in section IV of the table would bring the wheat data of that section into very close agreement with the Coleus data.)

The data of table 1, section III, demonstrate that the index of greater water-supplying power (B) has practically the same value for all soils, and that its variations exhibit no relation to the progressively increasing series of the water-holding-power values. Remembering that the soil series are arranged in the order of progressively increasing water-holding power, the truth of the last statement is clearly established by noting that the twelve soils, when arranged according to increasing values of this index, give the following series of soil numbers: 3, 9, 8, 2, 7, 4, 6, 10 (the last two are alike), 12, 11, 1, 5. There is surely no relation between the meanings of these figures and the order of their arrangement.

These average values of the greater water-supplying-power range from 1270 to 1534 and the average of averages is 1412. The maximum is 8.6 per cent greater than the mean and the minimum is 10 per cent smaller. The water contents corresponding to these supplying-power values, had they been determined, must have shown a progressive variation similar to that seen in the series of water-holding-power values (A).

When the index of the critical water-supplying-power-residue is divided by the corresponding index of the greater water-supplying power, giving the supplying-power-residue fraction (table 1, section V, D/B) we obtain another series of values that do not show any relation to the progressively increasing water-holding-power values, and that are to be considered as all practically alike. The last observation strengthens the conclusion that the critical water-supplying-power-residues (D) are to be taken as all alike, in spite of an apparently significant variation; they range only from a minimum of 0.5 to a maximum of 0.8 per cent of the corresponding greater supplying power, for Coleus, and from a minimum of 0.3 (or perhaps 0.4) to a maximum of 0.9 per cent for wheat. It is highly probable that our values for the greater supplying power are considerably too low, since they are based on a 30-minute period, which is probably too long for this instrument in such soils. Some 15-minute determinations on sand, loam and humus gave an average value of 3.157 gm., from which the derived value for a 2-hour period is 25.256 gm., or 2525.6 hundredths of a gram, instead of the mean value above derived from all soils, 1412 hundredths. Taking these last indications into account, we may therefore conclude that the index of water-supplying power at the inception of permanent wilting has a value, for both Coleus and wheat, of between 0.1 and 0.5 per cent (approximately) of the greater water-supplying power.

GENERAL CONCLUSIONS

It appears that the critical stage of wilting dealt with in this paper (the beginning of Briggs and Shantz's permanent wilting) occurs when the water-supplying power of the soil (as the latter becomes progressively drier after watering has been discontinued) has reached a certain low value, and that the soil-point method as here applied is adequate to determine this value within a reasonable degree of precision. For these instruments this value lies between 4 and 11 hundredths of a gram absorbed in a 2-hour period of exposure, the mean being 8.5. Of course this critical value refers to the two plants we have preliminarily tested and to the particular complex of non-soil-moisture conditions of the environment (chemical conditions of the soil, atmometric and light conditions of the air, etc.) with which we have worked. The critical value of the water-supplying power of the soil here stated *must not be thought of as a constant* for all kinds of plants and for all degrees of evaporation, etc. It will not prove to be a constant of this sort any more truly than did the "wilting coefficient" of Briggs and Shantz, which varies in a regular and predictable way, for any given soil and plant, with the evaporating power of the air that obtains during the period of wilting (5). This critical water-supplying power of the soil *does not vary*, however, with the physical make-up of the soil; it is the same for sand, loam and humus and for various mixtures of these. Its relation to soil temperature has not been studied.

In order to present a clear picture of our present conception of the relations involved in the advent of permanent wilting, we may trace briefly the march of pertinent affairs during the drying-out process that leads to this critical stage of wilting. Consider a healthy plant (as of Coleus), rooted in a pot of any kind of soil in which it will grow healthily, and suppose temperature and evaporation conditions that are not severe enough to prevent good growth when the soil is wet. Now suppose that water is supplied to saturate the soil and that watering is thereafter discontinued. The water-supplying power of the soil at this time may be represented by an index value of about 2000, for our soil-points and for a 2-hour period of exposure. As time goes on, the soil becomes gradually drier and the index value for the water-supplying power decreases. The plant at first continues to obtain all the water that its roots can absorb, and growth continues as previously. Sooner or later there comes a time, however, when the water-absorbing power of the plant is greater than the effective water-supplying power of the pot of soil. At this stage incipient drying becomes pronounced enough so that growth is retarded or checked. With the atmometric conditions remaining as they were, but with the water-supplying power of the soil continuing to diminish, cessation of growth is soon followed by some wilting. At first, the leaves appear wilted only for the driest part of the day, and recovery (perhaps even a renewal of growth) occurs for the night hours. As the index of soil-moisture-supplying power continues to decrease, there comes a time when the plant remains obviously wilted throughout the night. When this is true, either the evaporating power of the air is very high or the water-supplying power of the soil is very low, or both. Supposing the atmometric conditions to remain practically unchanged, however, with the same diurnal march as they have had previously, the various stages of progressive wilting each correspond to a certain value of the continually diminishing water-supplying power, and this value would be practically the same for all kinds of soils. Finally, the decreasing index of water-supplying power attains a value of about 8.5 (having started its decrease with a value of about 2000), for the general conditions of our experiments, and then permanent wilting begins. If the experiment is continued permanent wilting becomes progressively more pronounced as the moisture-supplying power goes on decreasing, and desiccation and the death of the plant eventually supervene.

Turning to the prospective value of the concept of water-supplying power and of instruments suitable for its direct measurement, it is clear that some sort of standardized instrument essentially similar to the soil-points used in our preliminary study will be a very great aid not only in physiological and ecological instrumentation but also in practical affairs, as in greenhouse culture, forest nursery work, and garden and agricultural operations. This instrument forms a much needed complement to the porous-cup atmometer, and when it becomes possible to employ the two together, along with soil and air thermometers, many of the present obstacles to progress in physiological ecology will disappear. For field work, the employment of such instrumentation as is suggested by our results will turn the attention of students away from the mechanical analysis of soils, the determination of their water contents, the measurement of rainfall, etc , and will bring into prominence the three directly effective conditional complexes (as far as water relations are concerned); namely, the evaporating power of the air, the evaporating power of radiation, and the water-supplying power of the soil. If these matters may receive the attention which they clearly deserve it may be possible to combine the atmometric index, the radio-atmometric index and the index of the water-supplying power of the soil, so as to obtain, at length, a valuable single index of the environmental-moisture complex as it affects plants.

In conclusion, we are very far from regarding the present contribution as a complete and satisfactory treatise on the problems with which we have dealt. The new apparatus and the methods of its use are as yet exceedingly crude, and time will be required for the accomplishment of many improvements that are as yet largely unthought of. Our paper is brought forward, in the present youthful stage of this sort of dynamic study, with the hope that others may appreciate the importance of pressing forward along the lines here suggested, to the end that physiology, physiological ecology, agriculture and forestry may become progressively more quantitative, more dyanmic and more precise.

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CARRIERS OF NITROGEN IN FERTILIZERS

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In the 5-year cereal rotation which has been conducted in duplicate on the Wooster and Strongsville farms of the Ohio Agricultural Experiment Station since 1894 at Wooster and since 1895 at Strongsville, plots 17, 21, 23, 24 and 30 have received equal quantities of phosphorus, potassium and nitrogen, the phosphorus being carried in acid phosphate on plots 17, 12, 23 and 24 and in acid phosphate and tankage on plot 30. The potassium has in all cases been carried in muriate of potash. The nitrogen has been carried in nitrate of soda on plot 17, in linseed oilmeal on plot 21, in dried blood on plot 23, in sulfate of ammonia on plot 24 and in tankage on plot 30. The same comparison has been repeated in the potato rotation at Wooster. Since 1900 half the land in the cereal rotation has been cross-dressed with ground limestone, applied at the rate of 2 tons per acre while preparing the land for corn. At Wooster the other half of the land has been left untreated, while at Strongsville it has been cross-dressed twice with raw phosphate rock the total application amounting to 1½ tons per acre.

In these experiments every third plot is left without any fertilizer or manure, and the increase due to the treatment is computed by comparing each fertilized plot with the two unfertilized plots between which it lies, on the assumption that changes in the natural fertility of a field are more likely to be gradual than abrupt.

THE CEREAL ROTATION AT WOOSTER

In tables 1 and 2 the results are shown in comparison both of the total yields of the crops and of the increase due to the treatments.

Table 2 shows that on this acid soil oats and wheat have given larger yields after nitrate of soda than after any other treatment, but corn and the hay crops have given the largest yields after tankage. The explanation seems to be that the nonacidulated phosphate in the tankage has been more favorable to the growth of clover—a fact very evident on inspection of the fields—than acid phosphate, and this improvement in the conditions for clover has been reflected in the timothy associated with it and in the corn immediately following.

Table 2 shows that on the limed land nitrate of soda has been the most effective carrier of nitrogen, with sulfate of ammonia a close second.

TABLE 2

Comparison of carriers of nitrogen; Wooster, cereal rotation, unlimed land

CROP	CARRIERS OF NITROGEN				
	Nitrate of soda	Linseed oilmeal	Dried blood	Sulfate of ammonia	Tankage
	Average yield per acre*				
Corn (bushels)	46.77	46 68	47.42	45.79	48.43
Oats (bushels)	54.81	50.53	51.11	51.46	51.32
Wheat (bushels)	27 09	25.38	23.83	24.41	26.50
Clover (pounds)	2,372	2,022	1,923	1,986	2,718
Timothy (pounds)	2,969	2,772	2,730	2,740	3,491
Annual produce (pounds)†	3,970	3,719	3,655	3,676	4,058
Annual value	$39 09	$36.56	$35.99	$36.09	$40.40
	Average increase per acre				
Corn (bushels)	22 34	21.73	22 10	19.57	20.51
Oats (bushels)	24.78	20 94	21 74	21 73	18.84
Wheat (bushels)	15 34	13 54	12.07	12.39	14.51
Clover (pounds)	1,281	965	844	842	1,435
Timothy (pounds)	858	679	597	512	1,076
Annual produce†	1,702	1,462	1,390	1,360	1,565
Annual value	$18 56	$16 19	$15 39	$14.83	$17.75

* Average of 17 crops of corn, 15 of oats, 14 of wheat, 16 of clover and 12 of timothy.

† The "annual produce," as given in this and following tables, includes both grain and straw or stover, while the "annual value" is computed only on the grains and hay, rating corn at $1.00 per bushel, oats at 75 cents, wheat at $2.00 and hay at $20.00 per ton. At these prices it is apparently immaterial, in these experiments, whether we use the total weight of produce, or the value of the grains and hay, as the common denominator for all the crops.

THE CEREAL ROTATION AT STRONGSVILLE

In table 3 is shown the outcome of this test at Strongsville for the 25 years of the experiment, 1905 to 1919, inclusive.

The uniformity shown in the total yields in this test is probably largely due to the apathy of this soil toward nitrogen fertilization.

In table 4 is shown the relative effect of nitrogen in the duplicate experiments at Wooster and Strongsville, in which the same crops are grown in succession, and plots of the same number receive the same treatment, in both kind and quantity of fertilizing materials.

Both nitrogen and potassium are comparatively ineffective on the Strongsville soil.

THE POTATOES-WHEAT-CLOVER ROTATION AT WOOSTER

In the 3-year rotation of potatoes, wheat and clover at Wooster, plot 20 receives its nitrogen in nitrate of soda; plot 21 in linseed oilmeal; plot 23 in dried blood; plot 24 in sulfate of ammonia and plot 33 in tankage. Plot 33 was not included in this test until 1897, and therefore the results are given in table 5 for the 23 years, 1897 to 1919.

The application of lime in this rotation was begun in 1903 but was not completed until 1913. It is not apparent, however, that the liming has materially altered the relative effectiveness of the different nitrogen carriers.

In this experiment the natural fertility of the land is so high that there is less margin for increase from fertilizing than in the tests above described. Repeating the comparison made in table 4, we get the results for the potato rotation as shown in table 6; computed for the entire period of the experiment.

TABLE 2

Comparison of carriers of nitrogen; Wooster, cereal rotation, limed land

	CARRIERS OF NITROGEN				
	Nitrate of soda	Linseed oilmeal	Dried blood	Sulfate of ammonia	Tankage
Average yield per acre*					
Corn (bushels)	54 64	53 98	54.06	55 35	51.42
Oats (bushels)	55 63	54 57	53.71	54 64	51.19
Wheat (bushels)	30.92	28.67	28 89	30 63	27.28
Clover (pounds)	3,496	3,068	3,140	3,374	3,397
Timothy (pounds)	4,596	4,039	4,057	4,365	4,381
Annual produce (pounds)	4,538	4,215	4,250	4,392	4,280
Annual value	$47 77	$44 66	$44.82	$47 00	$44.43
Average increase per acre					
Corn (bushels)	24 85	22 98	22.50	23 03	17.81
Oats (bushels)	21 47	19 25	18.24	18 75	14.32
Wheat (bushels)	15 84	12.68	13 02	14.73	11.88
Clover (pounds)	1,813	1,401	1,481	1,643	1,580
Timothy (pounds)	1,569	1,045	1,051	1,224	1,154
Annual produce (pounds)	2,160	1,700	1,737	1,868	1,592
Annual value	$21 29	$17 45	$17 51	$19.05	$15 93

* Average of 17 crops of corn, 15 of oats, 14 of wheat, 16 of clover and 12 of timothy.

TABLE 3

Comparison of carriers of nitrogen; Strongsville, 25-year average yield and increase

CROP	CARRIERS OF NITROGEN				
	Nitrate of soda	Linseed oilmeal	Dried blood	Sulfate of ammonia	Tankage
Average yield per acre					
Corn (bushels)	41.52	38.66	39.91	37.94	41.81
Oats (bushels)	52.38	53.00	53.54	54.10	51.29
Wheat (bushels)	20.78	21.05	21.12	20.87	20.71
Clover (pounds)	3,052	2,895	2,951	2,892	3,004
Timothy (pounds)*	2,180	2,261	2,277	2,203	2,096
Annual produce (pounds)	3,414	3,430	3,474	3,413	3,378
Annual value	$34 94	$34.41	$34.92	$34.24	$34 54
Average increase per acre					
Corn (bushels)	12.85	10.15	11.25	9.60	15.54
Oats (bushels)	14.52	12.72	12 95	14.07	14 71
Wheat (bushels)	9.63	9 30	9 15	9 25	10 03
Clover (pounds)	958	646	706	721	1,068
Timothy (pounds)	395	372	396	366	454
Annual produce (pounds)	1,040	922	917	918	1,119
Annual value	$11.31	$9 69	$10 06	$9.90	$12.37

* Timothy until 1914 and soybeans mown for hay since.

TABLE 4

Relative effect of nitrogen at Wooster and Strongsville; 25-year average value of increase per acre

PLOT	TREATMENT	ANNUAL VALUE OF INCREASE	
		Wooster	Strongsville
2	Acid phosphate	$7.75	$7.69
6	Acid phosphate and nitrate of soda	14.77	10.21
	Gain for nitrogen	$7.02	$2.52
8	Acid phosphate and muriate of potash	$11.83	$8.42
11	Acid phosphate, muriate of potash and nitrate of soda	18.14	11.24
	Gain for nitrogen	$6.31	$2.82

TABLE 5

Comparison of carriers of nitrogen; Wooster, potato rotation, 23-year average yield and increase

CROP	CARRIERS OF NITROGEN				
	Nitrate of soda	Linseed oilmeal	Dried blood	Sulfate of ammonia	Tankage
Average yield per acre					
Potatoes (bushels)...	169.13	160.45	160.62	161.98	158.63
Wheat (bushels)...	37.71	37.55	36.40	37.25	37.26
Clover (pounds)...	4,006	3,603	3,680	3,658	4,030
Annual value...	$94.87*	$90.53	$90.07	$91.02	$92.82
Average increase per acre					
Potatoes (bushels)...	39.90	36.76	37.50	39.73	31.65
Wheat (bushels)...	11.74	10.73	11.43	11.62	11.35
Clover (pounds)...	8.24	4.59	5.38	4.97	6.23
Annual value...	$23.87*	$20.94	$22.01	$22.87	$20.19

* Rating potatoes at $1.00 a bushel, wheat at $2.00, and hay at $20.00 a ton.

TABLE 6

Effect of nitrogen in potatoes-wheat-clover rotation at Wooster, 25-year average value of increase per acre

CROP	TREATMENT	ANNUAL VALUE OF INCREASE
2	Acid phosphate ...	$7.73
6	Acid phosphate and nitrate of soda ...	12.50
	Gain for nitrogen...	$4.77
8	Acid phosphate and muriate of potash...	$17.73
11	Acid phosphate, muriate of potash and nitrate of soda...	19.01
	Gain for nitrogen...	$1.28

THE TOBACCO-WHEAT-CLOVER ROTATION AT GERMANTOWN

At the southwestern test farm of the Experiment Station, at Germantown, Montgomery County, a 3-year rotation of tobacco, wheat and clover has been in progress since 1903. In this experiment certain plots have received the following treatment:

Plot	
8	Acid phosphate, muriate of potash, nitrate of soda
26	Same, with 1000 pounds of lime
16	Acid phosphate, muriate of potash, sulfate of ammonia
28	Same, with 1000 pounds of lime
18	Acid phosphate, muriate of potash, tankage
29	Same, with 1000 pounds of lime
22	Acid phosphate, nitrate of potash, nitrate of soda

The fertilizers have been calculated to carry 30 pounds of phosphorus, 75 pounds of potassium and 38 pounds of nitrogen in each case. On plots 18 and 29 all the nitrogen and the larger part of the phosphorus have been given in tankage, and on plot 22 all the potassium and the larger part of the nitrogen have been given in nitrate of potash. The fertilizers have all been applied to the tobacco crop, the wheat and clover following without further treatment.

The average yields obtained in this experiment during the 16 years, 1903–1918, are shown in table 7.

These results indicate a superiority of nitrate of soda over sulfate of ammonia on unlimed land, which disappears when the land is limed. The tobacco has shown a marked preference for either of the other carriers of nitrogen over tankage, a preference evident throughout the growth of the crop, but the wheat and clover do not speak so positively on this point. It would seem that the tankage becomes available too slowly for such a short-season crop as tobacco.

TABLE 7

Comparison of carriers of nitrogen; Germantown, average yields per acre

CROP	CARRIERS OF NITROGEN			
	Nitrate of soda	Sulfate of ammonia	Tankage	Nitrate of potash
Unlimed land				
	Plot 8	*Plot 16*	*Plot 18*	*Plot 22*
Tobacco (pounds)	1,227	1,095	960	1,131
Wheat (bushels)	26 63	25 82	26.16	25.15
Clover (pounds)	4,416	4,149	4,360	3,987
Annual values*	$93 82	$85.79	$79 97	$86 61
Limed land				
	Plot 26	*Plot 28*	*Plot 29*	
Tobacco (pounds)	1,069	1,082	959	
Wheat (bushels)	27.78	28 05	23.96	
Clover (pounds)	4,139	4,193	4,054	
Annual values*	$85 77	$86.78	$77.44	

* Rating tobacco at 15 cents a pound; wheat at $2.00 a bushel and hay at $20.00 a ton.

The plots in this test are arranged in blocks of 10 plots, the first, fourth, seventh and tenth plot in each block being left continuously unfertilized, and the increase due to the fertilizers is calculated on the assumption that variations in the yields of consecutive unfertilized plots are due to progressive variations in the soil. By this method of calculation the increases due to the separate treatment are found as shown in table 8.

TABLE 8

Comparison of carriers of nitrogen; Germantown, average increase per acre

CROP	CARRIERS OF NITROGEN			
	Nitrate of soda	Sulfate of ammonia	Tankage	Nitrate of potash
Increase on unlimed land				
	Plot 8	*Plot 16*	*Plot 18*	*Plot 22*
Tobacco (pounds)	656	623	506	600
Wheat (bushels)	14 09	14 14	15 03	14.67
Clover (pounds	1,632	1,368	1,629	1,509
Annual value	$47 63	$45 14	$40 75	$44.81
Increase on limed land				
	Plot 26	*Plot 28*	*Plot 29*	
Tobacco (pounds)	589	626	505	
Wheat (bushels)	17 11	17.46	13 57	
Clover (pounds)	1,907	1,934	1,798	
Annual value	$47 21	$49 39	$40.29	

TABLE 9

Comparison of carriers of nitrogen; annual values per acre at Wooster and Strongsville

EXPERIMENT	CARRIERS OF NITROGEN				
	Nitrate of soda	Linseed oilmeal	Dried blood	Sulfate of ammonia	Tankage
Value of total produce					
Wooster cereal rotation, unlimed	$39 09	$36 56	$35 99	$36 09	$40 40
Wooster cereal rotation, limed	47 77	44 66	44 82	47.00	44 43
Wooster potato rotation	94 87	90.53	90 07	91.02	92 82
Strongsville cereal rotation	34 94	34 41	34 92	34.24	34.54
Average	$54.17	$51 29	$51.45	$52.09	$53.05
Value of increase					
Wooster cereal rotation, unlimed	$18.56	$16.19	$15 39	$14.83	$17.75
Wooster cereal rotation, limed	21 29	17 45	17 51	19 05	15.93
Wooster potato rotation	23.87	20.94	22 01	22 87	20 19
Strongsville cereal rotation	11.31	9 69	10.06	9.90	12.37
Average	$18.76	$16 07	$16.24	$16 66	$16.56

The wheat and clover show a larger increase over the unfertilized yields on the limed than on the unlimed land except on the tankage plot, where the wheat falls behind.

Unless the land is exceptionally uniform, the comparison of increases, calculated by the method employed in these experiments, is usually more reliable than that of entire produce.

SUMMARY

In table 9 the annual values found in the experiments at Wooster and Strongsville are collected for convenient comparison.

These results show that, with only two exceptions in the forty comparisons, nitrate of soda has produced the largest yield, and this outcome is supported by the results at Germantown, except on the limed land, where the yields from sulfate of ammonia slightly exceed those from nitrate of soda.

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